Clean Water Act §319(h) Nonpoint Source Grant Program

Identify and Characterize Nonpoint Source Bacteria Pollution to Support Implementation of Bacteria TMDLs in the Oso Bay Watershed

TSSWCB Project 07-13

Revision 1
March 30, 2010

Quality Assurance Project Plan
Texas State Soil and Water Conservation Board

Prepared by
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Effective Period: Upon EPA approval to September 30, 2011

Questions concerning this quality assurance project plan should be directed to:

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Section A.1 Approval Sheet

Identify and Characterize Nonpoint Source Bacteria Pollution to Support Implementation of Bacteria TMDLs in the Oso Bay Watershed. (07-13)

United States Environmental Protection Agency, Region VI

_____ Date:_____
Donna Miller
USEPA Chief State/Tribal Programs Section

_____ Date:_____
Henry Brewer
USEPA Texas Nonpoint Source Project Manager

Texas State Soil and Water Conservation Board

_____ Date:_____
Mitch Conine
TSSWCB Project Manager

_____ Date:_____
Donna Long
TSSWCB Quality Assurance Officer

Texas A&M University-Corpus Christi

_____ Date:_____
Joanna Mott, Ph.D.
Project Leader

_____ Date:_____
Richard Hay, P.G.
Project Co-Leader

_____ Date:_____
La Donna Henson
Quality Assurance Officer

_____ Date:_____
Sergio Rodriguez
Field and Laboratory Supervisor

Section A.2 Table of Contents

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List of Acronyms and Abbreviations

ARP	Antibiotic Resistance Profile
AWRL	Ambient Water Reporting Limit
BST	Bacterial Source Tracking
CAR	Corrective Action Report
CBBEP	Coastal Bend Bays and Estuaries Program
CFU	Colony Forming Units of Bacteria
CLSI	Clinical Laboratory Standards Institute
COC	Chain of Custody
CRP	Texas Clean Rivers Program
CSU	Carbon Source Utilization
CWA	federal Clean Water Act
DO	Dissolved Oxygen
DQO	Data Quality Objectives
EML	Environmental Microbiology Laboratory at TAMU-CC
GPS	Global Positioning System
I-Plan	TMDL Implementation Plan
ID	Identification
NCCLS	National Committee for Clinical Laboratory Standards
NELAC	National Environmental Laboratory Accreditation Conference
NPS	Nonpoint Source
PL	Project Leader
QA	Quality Assurance
QAM	Quality Assurance Manual
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
QC	Quality Control
QPR	Quarterly Progress Report
RPD	Relative Percent Deviation
SM	Standard Methods for the Examination of Water and Wastewater, 21st Ed.
SOP	Standard Operating Procedure
SWQMIS	Surface Water Quality Monitoring Information System
TAMU-CC	Texas A&M University-Corpus Christi
TCEQ	Texas Commission on Environmental Quality
TGLO	Texas General Land Office
TMDL	Total Maximum Daily Load
TSSWCB	Texas State Soil and Water Conservation Board
USEPA	United States Environmental Protection Agency
USF	University of South Florida
USGS	United States Geological Survey
WWTF	Wastewater Treatment Facility

Section A.3 Distribution List

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

United States Environmental Protection Agency, Region VI

6WQ-AT
1445 Ross Avenue, Suite 1200
Dallas, TX 75202-2733

Name: Donna Miller
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Name: Joanna Mott, Ph.D.
Title: Project Leader

Name: Richard Hay, P.G.
Title: Project Co-Leader

Name: La Donna Henson
Title: TAMU-CC QAO

Name: Sergio Rodriguez
Title: Field and Laboratory Supervisor

Section A.4 Project/Task Organization

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

USEPA – Provides project oversight and funding at the federal level.

Henry Brewer, USEPA Project Officer
Responsible for managing the CWA §319(h) NPS Grant on the behalf of USEPA. Assists the TSSWCB in approving projects that are consistent with the management goals designated under the State's NPS Management Program and meet federal guidance. Coordinates the review of project work plans, QAPPs, draft deliverables, and works with the TSSWCB in making these items approvable. Fosters communication within USEPA by updating management and others, both verbally and in writing, on the progress of the State's program and on other issues as they arise. Assists in grant close-out procedures ensuring all deliverables have been satisfied prior to closing a grant.

TSSWCB – Provides project oversight and funding at the state level.

Mitch Conine, TSSWCB Project Manager
Maintains a thorough knowledge of work activities, commitments, deliverables, and time frames associated with project. Develops lines of communication and working relationships between TAMU-CC, TSSWCB, and USEPA. Tracks deliverables to ensure that tasks are completed as specified in the contract. Responsible for ensuring that the project deliverables are submitted on time and are of acceptable quality and quantity to achieve project objectives. Participates in the development, approval, implementation, and maintenance of the QAPP. Assists the TSSWCB QAO in technical review of the QAPP. Responsible for verifying that the QAPP is followed by TAMU-CC. Notifies the TSSWCB QAO of particular circumstances that may adversely affect the quality of data derived from the collection and analysis of samples. Enforces corrective action.

Donna Long, TSSWCB Quality Assurance Officer
Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB and USEPA participants. Responsible for verifying that the QAPP is followed by project participants. Determines that the project meets the requirements for planning, QA, QC, and reporting under the CWA §319(h) NPS Grant Program. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures.

TAMU-CC – performs project tasks as per contract and following the approved QAPP..

Joanna Mott and Richard Hay, TAMU-CC Project Leaders
Responsible for ensuring tasks and other requirements in the contract are executed on time and are of acceptable quality. Monitors and assesses the quality of work. Coordinates attendance at conference calls, training, meetings, and related project activities with TSSWCB. Responsible for writing and maintaining the QAPP in cooperation with the TAMU-CC QAO. Responsible for verifying the QAPP is followed and the project is producing data of known and acceptable quality. Notifies the TSSWCB Project Manager of particular circumstances that may adversely affect the quality of data derived from the collection and analysis of samples. Enforces corrective action. Responsible for developing, and providing TSSWCB with, project final report.

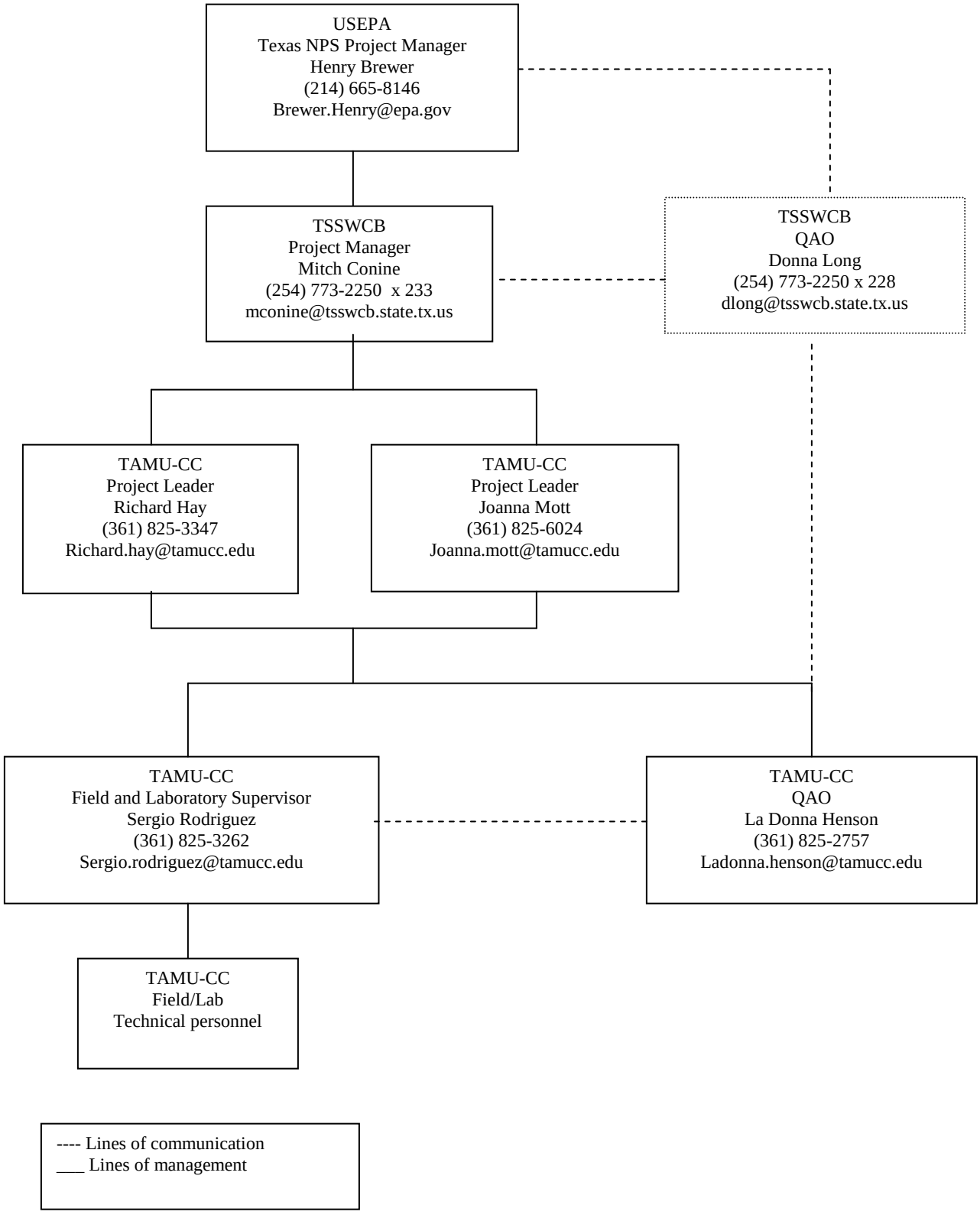
La Donna Henson, TAMU-CC Quality Assurance Officer
Responsible for coordinating development and implementation of the QA program. Participates in the planning, development, approval, implementation, and maintenance of the QAPP. Responsible for maintaining records of QAPP distribution, including appendices and amendments. Responsible for identifying, receiving, and maintaining project QA records. Responsible for coordinating with the TSSWCB QAO to resolve QA-related issues. Notifies the TAMU-CC Project Leaders and TSSWCB Project Manager of particular circumstances that may adversely affect the quality of data. Responsible for validation and verification of all data collected according to Table A.7.1 and QC specifications and acquired data procedures after each task is performed. Coordinates the research and review of technical QA material and data related to water quality monitoring system design and analytical techniques. Develops, facilitates, and conducts monitoring systems audits. Monitors the implementation of the EML QAM and the QAPP within the laboratory to ensure complete compliance with QA objectives as defined by the contract and in the QAPP. Conducts internal audits to identify potential problems and ensure compliance with written SOPs. Responsible for supervising and verifying all aspects of QA/QC in the laboratory. Performs validation and verification of data before data are evaluated to assess project objectives. Insures that all QA reviews are conducted in a timely manner from real-time review at the bench during analysis to final pass-off of data to the TAMU-CC PLs. Conducts laboratory inspections.

Sergio Rodriguez, TAMU-CC Field and Laboratory Supervisor
Responsible for supervising all aspects of the sampling and measurement of parameters in the field. Responsible for the acquisition of field (water, soils, sediment, fecal) samples and field data measurements in a timely manner that meet the DQOs specified in Section A.7, as well as the requirements of 0 through Section B.9. Responsible for field scheduling, staffing, and ensuring that staff are appropriately trained as specified in Section A.6 and Section A.8. Responsible for supervision of laboratory personnel involved in generating analytical data for this project. Responsible for ensuring that laboratory personnel involved in generating analytical data have adequate training and a thorough knowledge of the QAPP and all SOPs specific to the analyses or task performed

and/or supervised. Responsible for oversight of all operations, ensuring that all QA/QC requirements are met, and documentation related to the analysis is completely and accurately reported. Enforces corrective action, as required. Develops and facilitates laboratory systems audits.

TAMU-CC will provide copies of this QAPP and any amendments or appendices of this plan to each person on this list. TAMU-CC will document distribution of the QAPP and any amiendements and appendices, maintain this documentation as part of the projects's QA records, and will be available for review.

Figure A.4.1. Project Organization Chart



Section A.5 Problem Definition/Background

Oso Creek (Segment 2485A) is identified on the *2004 Texas Water Quality Inventory and 303(d) List* as impaired due to excessived bacteria. Results of a modeling study of bacteria loading for Oso Creek, submitted to TCEQ for use in the TMDL process, showed that loading occurs throughout the length of the creek, including the upper reaches and that there is "dry day" loading in addition to wet weather runoff and inflows. Modeling efforts demonstrated that the removal of the relatively small dry day loading could nearly achieve the geometric mean water quality standards in the creek; however, modeling work was unable to discern the source of the "dry day" loading. While there are several identified inflows downstream (stormwater, etc.) carrying runoff, the upper sections of the creek run through primarily rural agricultural row crop fields with no obvious sources of fecal bacteria. The creek is effluent driven, receiving water from the Robstown WWTF. The WWTF is permitted by TCEQ and is in compliance with effluent limits in the discharge permit. However, sampling of the creek showed elevated enterococci levels and loading is occurring in the upstream sections. A recently concluded study (TSSWCB Project 02-13; USGS 2008) which includes limited bacterial sampling of agricultural land runoff has indicated elevated levels of enterococci in this runoff.

Thus the previous studies to support the TMDL (monitoring data and modeling) have provided information on the levels of enterococci in the creek and bacteria loading for the TMDL but have not answered the key questions needed to finalize the TMDL or develop the I-Plan: what and where are the source(s) of the bacteria. In order for effective planning by stakeholders, the questions of where the bacteria are originating from in the upper portion of the creek needs to be answered.

This project will address both these issues through two investigations – one focused on the upper creek watershed and the possible types of bacteria (soil, sediment, subsurface flow) and the second focused on BST to determine the animal/human sources of the bacteria in the creek.

At a TMDL public stakeholder meeting (Feb. 8, 2007), a need for scientific studies to determine why crop and rangeland runoff concentrations are high so that appropriate management practices can be developed was identified. There was also discussion of the role and possible contribution of enterococci in the sediments.

Thus this project will provide critical information for understanding the bacteria loading in the Oso watershed to aid in the development of the creek TMDL and I-Plan.

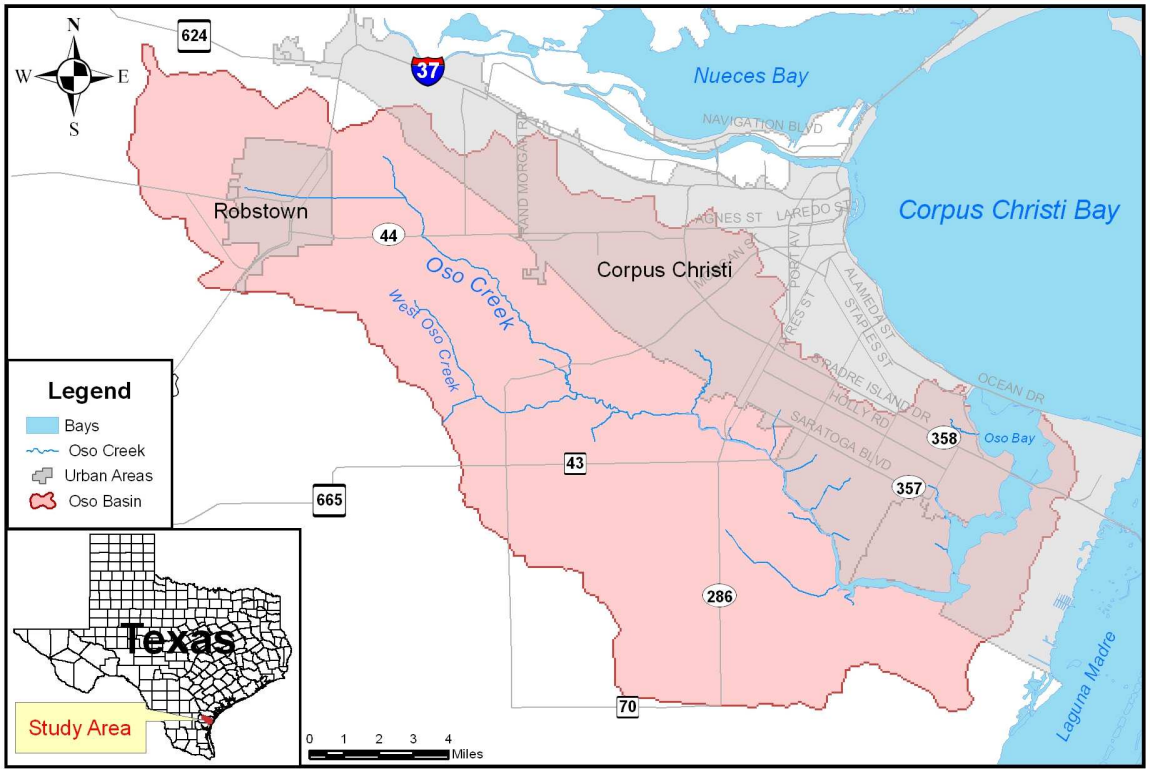


Figure A.5.1. Oso Basin study area.

Section A.6 Project/Task Description

The project will focus on the Oso Creek watershed to answer key questions that have arisen during the initial phase of the TMDL – what are the nonpoint sources of enterococci in the upper sections of the creek and what are the animal sources contributing to the loading. Animal sources of enterococci in Oso Creek will be determined using two library-dependent BST techniques – Carbon Source Utilization (CSU) and Antibiotic Resistance Profiling (ARP) of isolates. This information will also be of use for other similar watersheds (e.g., contributions of sediment and agricultural runoff).

The project has four objectives:

(1) Enterococci levels in the upper section of Oso Creek will be explained by identification of nonpoint sources of fecal contamination
(2) Enterococci levels in the upper sections of the creek, sediments and subsurface waters will be quantified
(3) Enterococci isolated from the creek under dry and wet conditions will be categorized by source type (human/non human, etc.)
(4) Additional data on enterococci levels in the creek will be collected

The tasks to address these objectives are as follows:

- A sampling strategy has been developed to elucidate the contributions of possible nonpoint sources of fecal bacteria (enterococci) with consultation and input from entities including TCEQ, the Coastal Bend Bays and Estuaries Program (CBBEP), the USGS, the Nueces River Authority, the Texas AgriLife Research Center at Corpus Christi (AgriLife Research), and local stakeholders (e.g., Cities of Corpus Christi and Robstown, local farmers, developers, discharge permit holders, homeowners). Field collection and lab analysis for enterococci will follow approved TCEQ procedures (TCEQ 2003) and approved USEPA lab analysis methods. Field sampling will include agricultural land runoff, dry soil sampling from representative in-field locations, and in-creek sediment and water sampling at multiple stations along the creek to identify any points of potential inflow and to determine the possible role of sediment as a contributor. Existing stations will be sampled quarterly to maintain a record of bacteria levels at those sites (18499, 18500, 18501). Sampling of subsurface water will also be conducted to examine the potential role of groundwater in the bacterial loading. Dr. Egon Weber, Director of the Center for Water Supply Studies at TAMU-CC will provide technical expertise (consultant) in examining the extent of contributions from groundwater discharge. Wells being constructed and maintained at a number of locations in the watershed through another project (funded by CBBEP) will be sampled at multiple depths, seasonally, under both dry and wet weather conditions. This project will analyze those samples for bacteria.

- Monitoring of the wells and in-stream creek water will continue and BST of the enterococci will be initiated to determine whether the creek is contaminated by human, livestock, or wildlife sources of bacteria. Enterococci isolates will be characterized using the Micrologᵀᴹ Microbial Identification System (Biolog 1994), which provides a species level identification and a CSU profile for each isolate. Speciation provides some information about sources as certain species are associated with specific animals. The existing Texas Known Source Library does not contain known source isolates of enterococci; however, a small library exists at TAMU-CC from a previous TGLO contract. These isolates will be incorporated iinto this project and will be supplemented with additional known source enterococci collected in this study in order to categorize the unknown source isolates by discriminant analysis. ARP will also be developed for each isolate to provide a composite data set with the CSU. While Texas BST work has focused on *E. coli* (as it is the indicator for freshwater bodies), for coastal (marine) waters where the indicator is enterococci it is more appropriate to use this group in TMDL studies. Although the upper creek is freshwater, the Oso Creek/Oso Bay TMDLs are based on enterococci as the segment includes marine and tidal sections. Enterococci have been used in previous studies in other states for BST work and can provide at least equivalent (and sometimes better) discrimination between sources. A subset of samples will also be analyzed for detection of the *esp* gene, which is a marker for human source enterococci. This will provide an additional level of confidence in the data. Detection of the *esp* gene is a library-independent BST method.

- Additional small scale studies of survival and re-growth in stream sediments and/or agricultural field soil will be conducted, dependent on the initial sampling results. All laboratory analyses involved in these studies will follow standard methods as referenced in this QAPP. A few sediment cores collected at a downstream station of the creek have contained enterococci but work has not been conducted upstream or in any depth.

- A final report will be prepared to include the results of the study for use in the development of the Oso Creek TMDL and I-Plan.

The project plan milestones for the project/task description of this section of the study are summarized in Table A.6.1.

Table A.6.1. Project Plan Milestones

Task	Project Milestones	Start[1]	End[2]
1	**Project Administration and Coordination**		
1.1	Submit Quarterly Progress Reports	Month 1	Month 51
1.2	Order supplies, accounting, quarterly reimbursement submittals	Month 1	Month 51
1.3	Technical oversight of lab	Month 1	Month 51
1.4	Attendance and participation at Oso Watershed stakeholder meetings, other appropriate meetings	Month 1	Month 51
2	**Sampling Design**		
2.1	Meet with local entities to determine potential bacteria sources	Month 1	Month 9
2.2	Prepare field sampling plan	Month 1	Month 9
3	**Quality Assurance Project Plan**		
3.1	Write and submit QAPP, revise as needed	Month 9	Month 18
4	**Field Sampling and Lab Analysis to Identify Sources of Enterococci**		
4.1	Sampling of subsurface waters under dry and wet conditions	Month 20	Month 44
4.2	Sampling at historic stations	Month 19	Month 47
4.3	Sampling of sediments, soils	Month 20	Month 44
4.4	Lab testing of soils and sediments	Month 36	Month 44
5	**Bacteria Source Tracking to Determine Animal Sources of Contamination**		
5.1	Develop ARA and CSU library of known source isolates	Month 27	Month 41
5.2	Collection of samples for unknown source isolates	Month 31	Month 41
5.3	Develop CSU and ARP profiles for unknown isolates	Month 31	Month 43
5.4	Perform statistical analysis using profiles	Month 42	Month 47
5.5	Esp gene analysis	Month 34	Month 41
6	**Completion and Submission of Final Report**		
6.1	Complete and submit rough draft of report	Month 48	Month 50
6.2	Revise and submit final report	Month 50	Month 51

[1] Month 1 = October 2007.(assumes start of month shown)
[2] Month 51 = December 2011 (assumes end of month shown).

Revisions to the QAPP

Until the work described is completed, this QAPP shall be revised as necessary and reissued at least annually, or revised and reissued within 120 days of significant changes, whichever is sooner. If the entire QAPP is current and valid, the document may be reissued by certifying that the plan is current and including a new copy of the signed approval page. The approved version of the QAPP shall remain in effect until revised versions have been approved.

Expedited Changes

Expedited changes to the QAPP should be approved before implementation to reflect changes in project organization, tasks, schedules, objectives, and methods, address deficiencies and non-conformance, improve operational efficiency and accommodate unique or unanticipated circumstances. Requests for expedited changes are directed from the TAMU-CC PLs to the TSSWCB Project Manager in writing. They are effective immediately upon approval by the TSSWCB Project Manager and QAO and the USEPA Project Manager.

Justifications, summaries, and details of expedited changes to the QAPP will be documented and distributed to all persons on the QAPP distribution list. Expedited changes will be reviewed, approved, and incorporated into a revised QAPP during the annual revision process or within 120 days of the initial approval in cases of significant changes.

Section A.7 Quality Objectives and Criteria for Data Quality

The objective of this section is to ensure that data collected meets the DQOs of the project

The project will focus on the Oso Creek watershed to answer key questions that have arisen during the initial phase of the TMDL – what are the nonpoint sources of enterococci in the upper sections of the creek and what are the animal sources contributing to the loading. Animal sources of enterococci in Oso Creek will be determined using two library-dependent BST techniques – Carbon Source Utilization (CSU) and Antibiotic Resistance Profiling (ARP) of isolates. This information will also be of use for other similar watersheds (e.g., contributions of sediment and agricultural runoff).

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(4) Additional data on enterococci levels in the creek will be collected

The measurement performance criteria to support the project objectives are specified in Table A.7.1.

Routine grab samples will be collected quarterly at three historical stations following *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). During routine sampling, measurements of dissolved oxygen (DO), conductivity, pH, salinity, stream flow, and water temperature will be obtained in situ. Water samples will be analyzed for Enterococcus. Sediment and soil samples will be collected using corers ,and for soils only, preparing composite samples for analysis.

The BST part of this project involves the measurement of non-routine parameters. Methods for these analyses used have been published and/or approved in previous QAPPs as follows. Enterococci will be isolated from water samples following USEPA methods (USEPA 2000). Colonies will be transferred, confirmed as *Enterococcus*, speciated and characterized by CSU using the MicroLog™ Microbial Identification System (Biolog, Inc., 3938 Trust Way, Hayward, CA 94545) following the MicroLog™ System Release 4.0 User Guide (Biolog, 1999). Fecal samples will be collected as approved in a special study plan “Application of antibiotic resistance patterns to differentiate sources of *E. coli* in coastal waters of Texas” (2000), prepared by Dr. Mott for TCEQ and as detailed in Section B.2. ARP will follow published clinical standards as described in the following CLSI documents: Performance Standards for Antimicrobial Disc

Susceptibility Tests Approved Standard-Ninth Edition, CLSI document M2-A9 (2006); Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals Approved Standard-Third Edition, CLSI document M31-A3 (2008); and Performance Standards for Antimicrobial Susceptibility Testing Eighteenth Informational Supplement, CLSI document M100-S18 (2008). The *esp* gene analysis will follow the USF-TAMU-CC SOP from a protocol provided by Dr. Harwood and the 2006 paper:. McQuaig, S.M., Scott, T.M., Harwood, V.J., Farrah, S.R. and Lukasik, J.O. (2006) Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. *Appl Environ Microbiol* **72**, 7567-7574. The TAMU-CC SOPs for all three of these methods are included in the Appendix D.

Table A.7.1. Measurement Performance Specifications.

NA = Not applicable

PARAMETER	UNITS	MATRIX	METHOD	PARAMETER CODES	AWRL	Lab Reporting Limits	Recovery at Reporting Limits	PRECISION (RPD of LCS/LCSD)	BIAS (% Rec. LCS/LCSD mean)	Laboratory Performing Analysis
Field Parameters										
pH	pH units	Water	EPA 150.1 & TCEQ SOP	00400	NA	NA	NA	NA	NA	Field
DO	mg/L	Water	EPA 360.1 & TCEQ SOP	00300	NA	NA	NA	NA	NA	Field
Conductivity	uS/cm	Water	EPA 120.1 & TCEQ SOP	00094	NA	NA	NA	NA	NA	Field
Water Temperature	°C	Water	EPA 170.1 & TCEQ SOP	00010	NA	NA	NA	NA	NA	Field
Secchi Disk Transparency	Meters	Water	TCEQ SOP	00078	NA	NA	NA	NA	NA	Field
Days since last significant rainfall	Days	NA	TCEQ SOP	72053	NA	NA	NA	NA	NA	Field
Instantaneous Flow	Cfs	Water	TCEQ SOP	00061	NA	NA	NA	NA	NA	Field
Flow measurement method	1-gage 2-electric 3-mechanical 4-weir/flume 5-doppler	Water	TCEQ SOP	89835	NA	NA	NA	NA	NA	Field
Flow Severity	1-no flow 2-low 3-normal 4-flood 5-high 6-dry	Water	TCEQ SOP	01351	NA	NA	NA	NA	NA	Field
Total water depth	Meters	Water	TCEQ SOP	82903	NA	NA	NA	NA	NA	Field
Salinity	Ppt	Water	SM 2520 & TCEQ SOP	00480	NA	NA	NA	NA	NA	Field
Flow estimate	Cfs	Water	TCEQ SOP	74069	NA	NA	NA	NA	NA	Field
Maximum pool width	Meters	Water	TCEQ SOP	89864	NA	NA	NA	NA	NA	Field

PARAMETER	UNITS	MATRIX	METHOD	PARAMETER CODES	AWRL	Lab Reporting Limits	Recovery at Reporting Limits	PRECISION (RPD of LCS/LCSD)	BIAS (% Rec. LCS/LCSD mean)	Laboratory Performing Analysis
Tide stage	1-low 2-falling 3-slack 4-rising 5-high	Water	TCEQ SOP	89972	NA	NA	NA	NA	NA	Field
Rainfall (inches past 1 day)	Inches	NA	TCEQ SOP	82553	NA	NA	NA	NA	NA	Field
Rainfall (inches past 7 days)	Inches	NA	TCEQ SOP	82554	NA	NA	NA	NA	NA	Field
Water color	1-brown 2-red 3-reen 4-black 5-clear 6-other	Water	TCEQ SOP	89969	NA	NA	NA	NA	NA	Field
Water odor	1-sewage 2-oily/chemical 3-rotten eggs 4-musky 5-fishy 6-none 7-other	Water	TCEQ SOP	89971	NA	NA	NA	NA	NA	Field
Water surface	1-calm 2-ripple 3-wave 4-whitecap	Water	TCEQ SOP	89968	NA	NA	NA	NA	NA	Field
Air temperature	°C	Air	TCEQ SOP	00020	NA	NA	NA	NA	NA	Field
Wind intensity	1-calm 2-slight 3-moderate 4-strong	Water	TCEQ SOP	89965	NA	NA	NA	NA	NA	Field
Wind direction	1-north 2-south 3-east 4-west 5-northeast 6-southeast 7-northwest 8-southwest	Air	TCEQ SOP	89010	NA	NA	NA	NA	NA	Field

PARAMETER	UNITS	MATRIX	METHOD	PARAMETER CODES	AWRL	Lab Reporting Limits	Recovery at Reporting Limits	PRECISION (RPD of LCS/LCSD)	BIAS (% Rec. LCS/LCSD mean)	Laboratory Performing Analysis
Present weather	1-clear 2-partly cloudy 3-cloudy 4-rain	NA	TCEQ SOP	89966	NA	NA	NA	NA	NA	Field
Laboratory Parameters										
Enterococcus	CFU/100 mL	Water	EPA Method 1600	31649	1.0	1.0	NA	3.27 ΣRlog/n*	NA	TAMU-CC
Enterococcus	CFU/gdw	Soil/sediment	EPA Method 1600**	NA	1.0	1.0	NA	3.27 ΣRlog/n*	NA	TAMU-CC
Enterococcus	NA – no enumeration	Known fecal	TAMU-CC SOP	NA	NA	NA	NA	NA	NA	TAMU0CC
CSU profile	Color intensity	Culture	Biolog/TAMU-CC SOP	NA	NA	NA	NA	NA	NA	TAMU-CC
Enterococcus speciation	Species	Culture	Biolog/TAMU-CC SOP	NA	NA	≥90% probability (Biolog software)	NA	NA	NA	TAMU-CC
ARA profile	Zone diameter mm	Water/soil/ sediment	CLSI Standard/TAMU-CC SOP	NA	NA	NA	NA	NA	NA	TAMU-CC
Esp gene	NA (presence/ absence)	Water	USF/TAMU-CC SOP	NA	NA	NA	NA	NA	NA	TAMU-CC

* Based on precision calculation method as described in Standard Methods, 21st Edition, Section 9020-B, "QA/QC - Intralaboratory QC Guidelines." This criterion applies to bacteriological duplicates with concentrations >10 org/100 mL.

** Method 1600 will be used following pre-treatment either using a standard dilution series followed by plating or shaking with a dispersant followed by filtration as per Soils Science Society of America (SSSA). "Methods of Soil Analysis Part 2 – Microbiological and Biochemiscal Properties". SSSA Book Series 5. 1994.

le; mg/L = milligrams per liter; col = colonies; mL = milliliters; m/s = meters per second; µS/cm = microsiemens per centimeter; ft = feet; m = meters; °C = degrees Celsius, gdw= grams dry weight

References for Table A7.1:

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF), "Standard Methods for the Examination of Water and Wastewater," 21st Edition, 2005

Biolog. 1999. MicroLog™ System Release 4.0 User Guide.

CLSI. 2006. Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard-Ninth Edition. CLSI document M2-A9.

CLSI. 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Third Edition. CLSI document M31-A3.

CLSI.. 2008. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. CLSI document M100-S18

McQuaig, S.M., Scott, T.M., Harwood, V.J., Farrah, S.R. and Lukasik, J.O. (2006) Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. Appl Environ Microbiol **72**, 7567-7574.

Soil Science Society of America (SSSA). "Methods of Soil Analysis Part 2 – Microbiological and Biochemiscal Properties". SSSA Book Series 5. 1994.

TCEQ SOP – *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008) or subsequent editions.

United States Environmental Protection Agency (USEPA), "Methods for Chemical Analysis of Water and Wastes," Manual #EPA-600-4-79-020

Ambient Water Reporting Limits and Laboratory Reporting Limits

Ambient water reporting limits, or AWRLs, are the specifications at or below which data for a parameter must be reported to be compared with the freshwater screening criteria. The AWRLs specified in Table A.7.1 are the program-defined reporting specifications for each analyte and yield data acceptable to meet the project objectives. The limit of quantitation (LOQ—formerly known as the reporting limit) is the minimum level, concentration, or quantity of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence. LOQ is not applicable for bacteria.

The laboratory is required to meet the following:

- The laboratory's reporting limit for each analyte must be at or below the AWRL as a matter of practice.
- The laboratory must demonstrate its ability to quantitate at its LOQ for each analyte by running an LOQ check standard for each batch of samples analyzed (not applicable for bacteria).

Acceptance criteria are defined in Table A.7.1.

Precision

Precision is the degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves. It is a measure of agreement among replicate measurements of the same property, under prescribed similar conditions, and is an indication of random error.

Laboratory precision is assessed by comparing replicate analyses of laboratory control samples in the sample matrix (i.e.-deionized water, sand, commercially available tissue) or sample/duplicate pairs in the case of bacterial analysis. Precision results are compared against measurement performance specifications and used during evaluation of analytical performance. Program-defined measurement performance specifications for precision are defined in Table A.7.1.

For the BST analyses control limits for laboratory control standard/laboratory control standard duplicates are specified in software associated with each technique to be used – MicroLog™ Microbial Identification System provides a % similarity of each isolate with known bacteria in the Biolog database, and BIO-MIC (for ARP analysis) follows CLSI standards, which include specifications for duplicate analyses. The *esp* gene analysis provides a presence/absence result and utilizes a positive control culture (*Enterococcus faecium*).

Representativeness

The data collected as routine grab samples will be considered representative of the target population or phenomenon to be studied. The representativeness of the data is dependent on 1)

the sampling locations, 2) the number of samples collected, 3) the number of years and seasons when sampling is performed, 4) the number of depths sampled, and 5) the sampling procedures. Site selection and sampling of pertinent media (i.e., water, soil, sediment, fecal material) and use of only approved analytical methods will assure that the measurement data represent the population being studied at the site. Data may be collected during varying regimes of weather and flow, data collection will be targeted toward both ambient conditions and storm events, representing water quality at high and low flow conditions. The goal for meeting total representation of the water body will be tempered by the availability of time, site assessibility, and funding. Representativeness will be measured with the completion of sample collection in accordance with the approved QAPP.

Comparability

Confidence in the comparability of data sets for this project is based on the commitment of project staff to use only approved sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as described in this QAPP. Comparability is also guaranteed by reporting data in standard units, by using accepted rules for rounding figures, and by reporting data in a standard format as specified in Section B10.

Completeness

The completeness of the data is a measure of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data should be available. However, the possibility of unavailable data due to accidents, weather, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project that 90% data completion is achieved.

An additional element of completeness is involved with BST. Some isolates may lose viability while being stored or transferred in the laboratory, and some will not confirm as *Enterococcus* spp. using the Biolog system. For example, some of the known source Enterococcus isolates currently frozen and in storage, for which CSU profiles already exist and to be used to expand the library for this study, may either not be located or will not re-grow, so the goal that ARA profiles will be developed for all of these isolates is not attainable. Every effort to obtain profiles for these isolates will be made. The sources of Enterococcus isolates which do not match those from a library of known sources cannot be identified. In all BST studies a source cannot be identified with acceptable confidence for a portion of the Enterococcus isolates or only a general grouping can be achieved with a level of confidence. This is a function of 1) the size of the library relative to the true diversity of Enterococcus in the watershed 2) the ability of the method to distinguish sources with acceptable confidence and 3) the abundance of Enterococcus strains that colonize multiple sources and thus cannot be used to uniquely identify a source.

Section A.8 Special Training Requirements/Certifications

Field personnel will receive training in proper sampling and field measurements. Before actual sampling or field analysis occurs, they will demonstrate to the TAMU-CC QAO or designee their ability to properly calibrate field equipment and perform field sampling and analysis procedures. Training will be documented and retained in the TAMU-CC personnel file and will be made available during a monitoring systems audit.

Laboratory analysts have a combination of experience, education, and training to demonstrate a knowledge of their function. Each laboratory analyst will have a demonstration of capability (DOC) on record for each test that the analyst performs. The initial DOC should be performed before analyzing samples and annually thereafter. For cases in which an analyst has been analyzing samples before an official certification of capability has been generated, a certification statement is made part of the training record to document the analyst's initial on the job training. Annual DOCs are a part of analyst training thereafter.

Section A.9 Documents and Records

The document and records that describe, specify, report, or certify activities, requirements, procedures, or results for this project and the items and materials that furnish objective evidence of the quality of items or activities are listed in Table A.9.1.

Hard copies of all field data sheets, general maintenance records, COC forms, laboratory data entry sheets, field data entry sheets, calibration logs, and CARs will be maintained on file by TAMU-CC for at least five years. In addition, TAMU-CC will maintain electronic forms of all project data for at least five years. Examples are presented of field data sheets in Appendix B, a COC form in Appendix C, and a CAR form in Appendix A.

QPRs will be produced electronically for the TSSWCB and will note activities conducted in connection with audits of the monitoring program, items or areas identified as potential problems, and any variations or supplements to the QAPP. CARs will be utilized when necessary (Appendix A). CARs will be maintained in an accessible location for reference at TAMU-CC. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP, when appropriate.

Individuals listed in Section A3 at TAMU-CC will be notified of approval of the most current version of the QAPP by the TAMU-CC PLs. The TAMU-CC PLs will make available to the laboratory and field personnel the most recent version of the QAPP. Current copies of the QAPP will be kept on file for all individuals on the TAMU-CC EML distribution list.

The final project report will be produced electronically and as a hard copy and files used to produce the final report will be saved electronically by TAMU-CC for at least five years.

Table A.9.1. Project Documents and Records.

Document/Record	Location	Retention	Form
QAPP, amendments, and appendices	TAMU-CC	5 years	Paper/Electronic
QAPP distribution documentation	TAMU-CC	5 years	Paper
Field data sheets	TAMU-CC	5 years	Paper
Field Demonstration of Capability records	TAMU-CC	5 years	Paper
Field equipment calibration/maintenance logs	TAMU-CC	5 years	Paper
Field SOPs	TAMU-CC	5 years	Paper
COC records	TAMU-CC	5 years	Paper
Field corrective action documentation	TAMU-CC	5 years	Paper
Laboratory sample reception logs	TAMU-CC	5 years	Paper
Laboratory QA manuals	TAMU-CC	5 years	Paper
Laboratory SOPs	TAMU-CC	5 years	Paper
Laboratory Demonstration of Capability records	TAMU-CC	5 years	Paper
Laboratory instrument readings/ printouts	TAMU-CC	5 years	Paper/Electronic
Laboratory data reports	TAMU-CC	5 years	Paper/Electronic
Laboratory data verification for integrity, preicison and validation	TAMU-CC	5 years	Paper
Laboratory equipment maintenance logs	TAMU-CC	5 years	Paper
Laboratory equipment calibration records	TAMU-CC	5 years	Paper/Electronic
Laboratory corrective action documentation	TAMU-CC	5 years	Paper
QPRs/final report/data	TAMU-CC & TSSWCB	3 years	Paper/Electronic

Laboratory Data Reports

Data reports from the laboratory will report the test results clearly and accurately. The test report will include the information necessary for the interpretation and validation of data and will include the following:

- name and address of the laboratory
- name and address of the client
- a clear identification of the sample(s) analyzed
- identification of samples that did not meet QA requirements and why (i.e., holding times exceeded)
- date of sample receipt
- sample results
- field split results (as applicable)
- a name and title of person accepting responsibility for the report
- project-specific QC results to include precision of LCS/LCSD pairs, equipment, trip, and field blank results (as applicable)
- narrative information on QC failures or deviations from requirements that may affect the quality of results.

All electronic data are backed up on an external hard drive weekly, compact disks monthly, and are simultaneously saved in an external network folder and the computer's hard drive. A blank CAR is presented in Appendix A, and a blank COC form is presented in Appendix C.

Section B

Section B.1 Sampling Process Design (Experimental Design)

Sample Design Rationale

The sample design is based on the project goals to determine the nonpoint sources of enterococci in the upper sections of the creek and the animal sources contributing to the loading. The environmental data collected under this QAPP must be collected and evaluated with a high degree of confidence such that the data are scientifically valid, of known quality, and legally defensible. Water, sediment and fecal samples are critical to the study.

Nonpoint sources of enterococci

Analysis of field samples and laboratory experiment samples will occur over the duration of this project. The first year of sampling will provide a synoptic dataset of the study area that will provide information on potential sources of enterococci and allow optimization of sampling resources for follow-up sampling and/or laboratory experiments by focusing on those areas identified as having high enterococci concentrations. Samples will be collected from a variety of potential sources including sediments, soils, sub-surface and groundwater as described below to evaluate contributions from these sources. Based on these results additional sampling will occur, with focused small-scale laboratory experiments if appropriate.

Note: the Nueces River Authority (NRA) conducts routine CRP monitoring at stations 13028 (Oso Creek at at SH 286 South of Corpus Christi) and 13440 (Oso Bay at Padre Island Drive (SH 358) quarterly (FY2009) (http://cms.lcra.org/).

a) Surface water samples at historic stations

To provide an updated database for historical stations sampled during previous TMDL studies, field water quality parameters (specific flow rate, specific conductance, dissolved oxygen, pH, salinity, and water temperature as listed in Table A.7.1) will be measured at three (3) historic TCEQ water quality monitoring sites (Figure B.1.1, Table B.1.1; individual maps: 18499 Figure B.1.2, 18501 Figure B.1.3, and 18500 Figure B.1.4) quarterly. Additionally, for each event two water samples will be collected from each of these sites for analysis of Enterococcus levels (Table B.1.7). This will continue for a total of ten (10) events during this project. Site(s) that are dry or with pooled water will be noted on the field data sheet and will not be sampled. In order to obtain representative results, ambient water sampling will occur on a routine schedule following QAPP approval through spring 2010, capturing dry and runoff-influenced events at their natural frequency. There will be no prejudice against rainfall or high flow events, except that the safety of the sampling crew will not be compromised in case of lightning or flooding. If, near the end of the study, the TSSWCB PM and TAMU-CC PLs agree that the sampling has not achieved good representativeness of typical conditions, the final sampling event(s) may be restricted to target a

particular environmental condition (e.g., rainfall). Such a modification in the sampling design would require an amendment to the QAPP.

Table B.1.1. Locations of Historic Water Quality Sampling Stations.

Station_ID(TCEQ ID)	Description	LAT	LON
18499	Oso Creek at SH 44	27.783250	-97.592430
18501	West Oso Creek at FM 665	27.709360	-97.554220
18500	Oso Creek at FM 665	27.729470	-97.523570
13029	Oso Creek at FB 763	27.711111	-97.501663
20559	Ditch on US 77 near Robstown WWTP	27.800060	-97.646530

Figure B.1.1. Historic TCEQ Water Quality Sampling Stations on Oso Creek and Oso Bay.

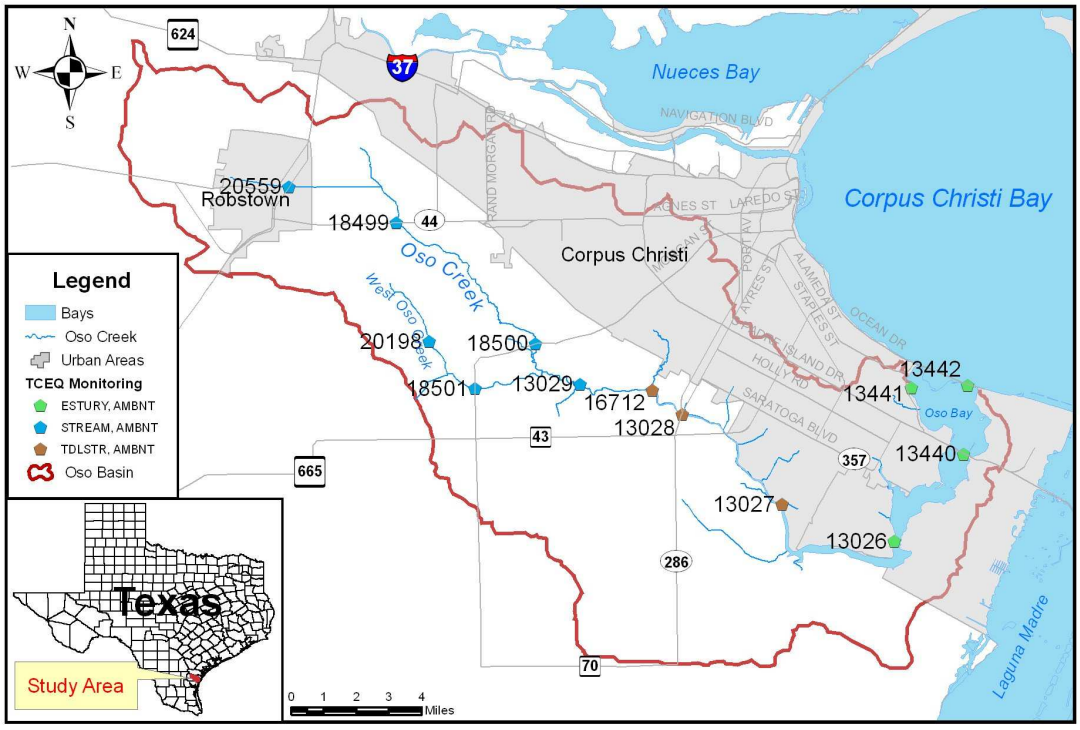

Figure B.1.2. Historic TCEQ Monitoring Station 18499.

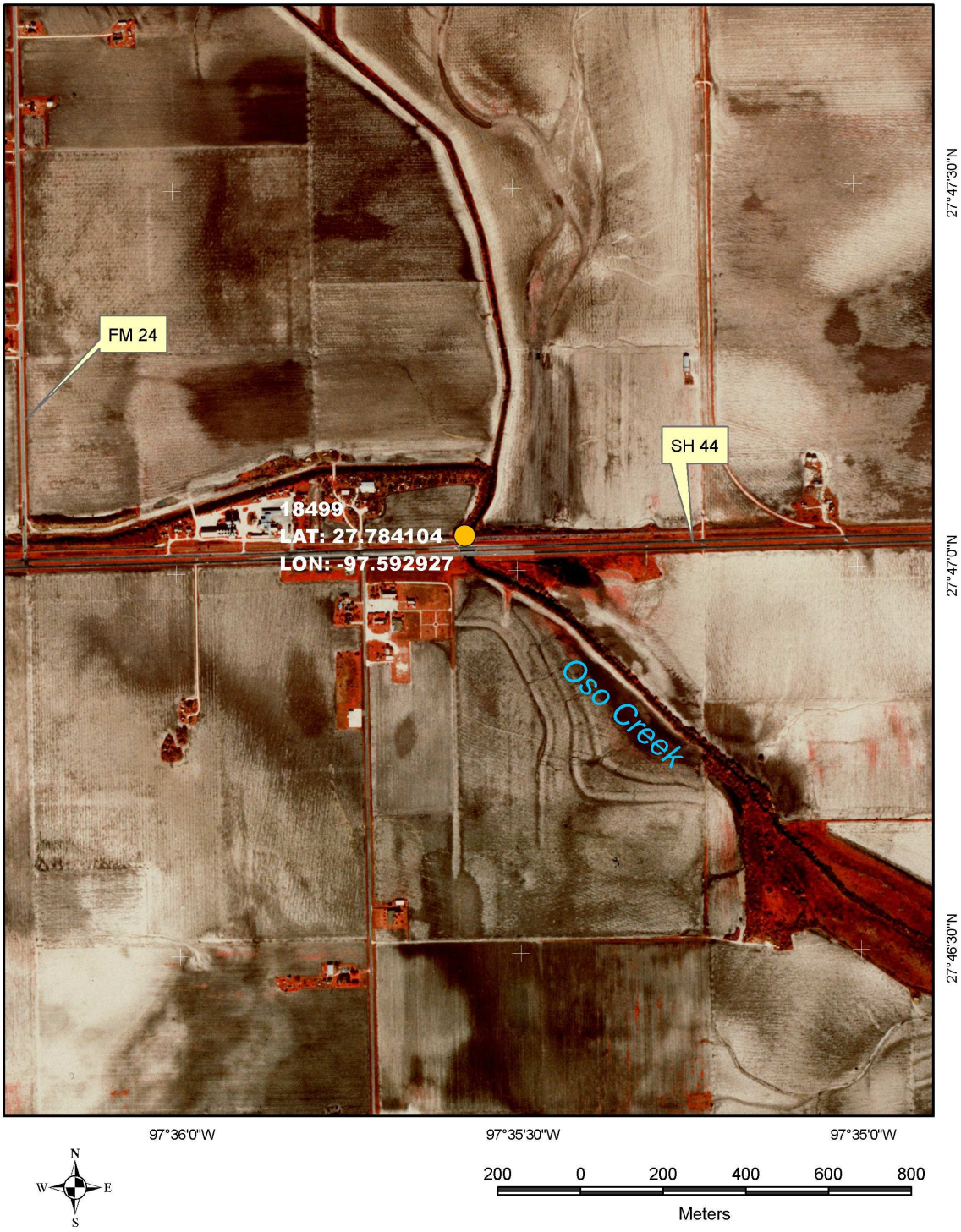

Figure B.1.3. Historic TCEQ Monitoring Station 18501.

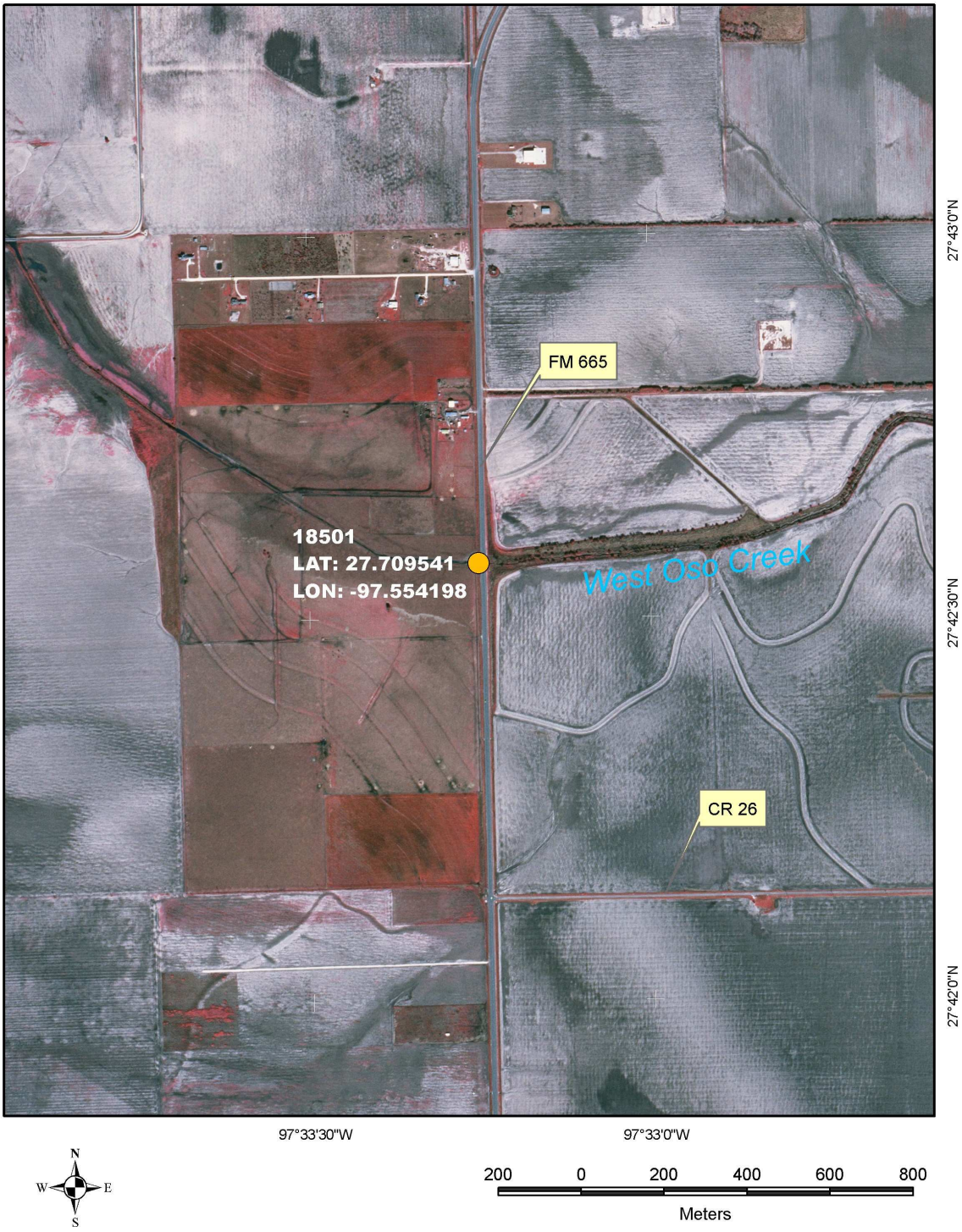

Figure B.1.4. Historic TCEQ Monitoring Station 18500.

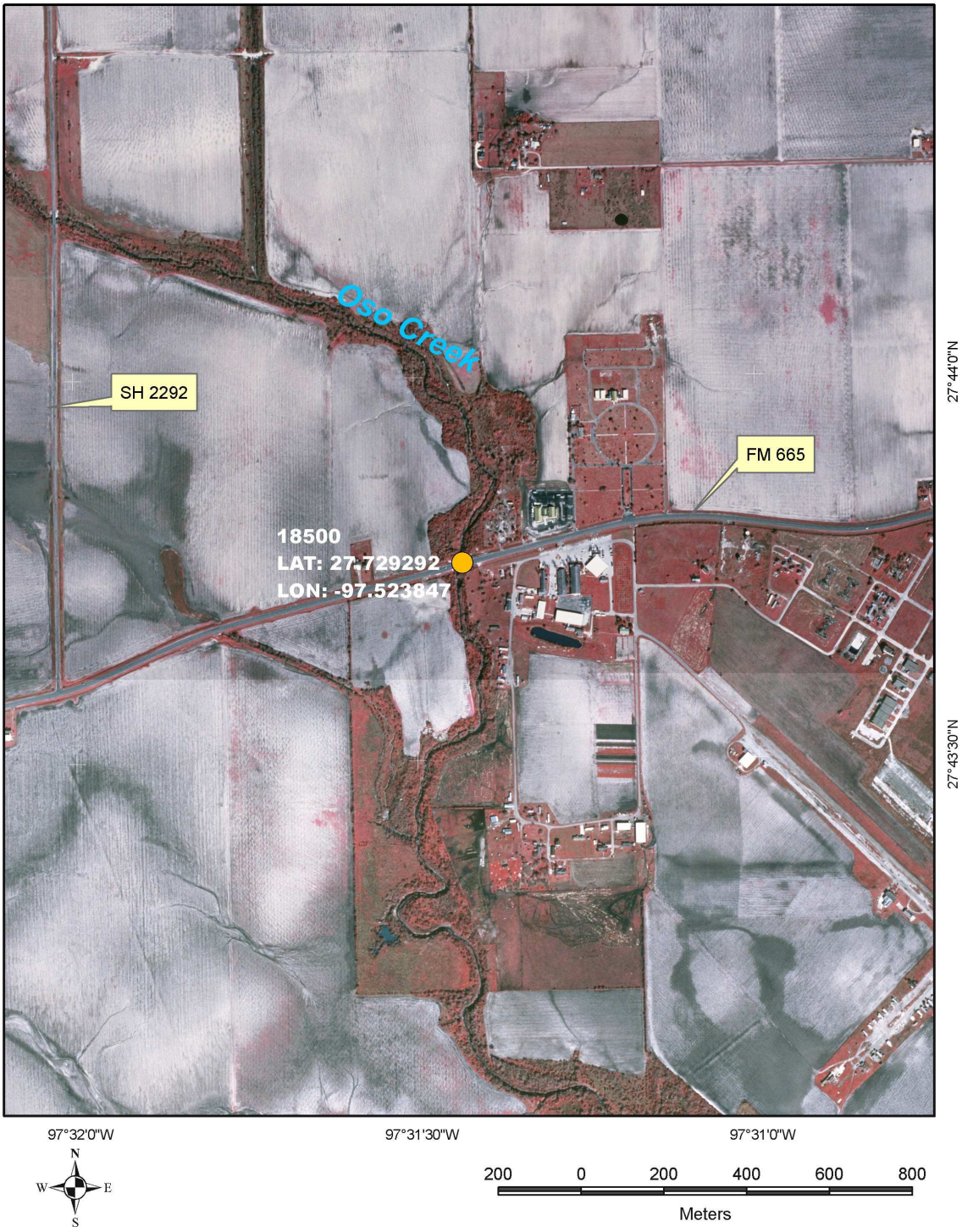

Figure B.1.5. Historic TCEQ Monitoring Station -20559.

b) Groundwater samples

Water samples will be collected from ten (10) groundwater wells established in pairs (one shallow – drilled to water table, and one deep – water table plus ~10 feet depending on subsurface stratigraphy) near historic TCEQ monitoring sites (18501, 18500, 18499 and 13029) and near agricultural NPS stations from previous and on-going AgriLife Research projects (TSSWCB projects 02-13 and 07-07) where historic data is available (Table B.1.2 and Figure B.1.6 sites 13, 14, 16, 25 and 26). The groundwater samples will be analyzed for *Enterococcus*. Two samples will be collected from each well quarterly during the first year of sampling, with an additional sampling event occurring between 5-10 days after a rainfall, resulting in 100 total samples. Continued sampling on a reduced schedule will depend on initial results.

c) Water and sediment samples

Water and sediment samples will be collected at five (5) monitoring locations established: immediately upstream from historic monitoring sites (18501, 18500, 18499); immediately upstream from the USGS gage station (gage #08211520) on Merritt Road where high concentrations have been measured in previous and on-going agricultural NPS studies (TSSWCB projects 02-13 and 07-07); and one location immediately upstream from a targeted monitoring point S7 (TCEQ station 20599, established during a recent TMDL study downstream of the Robstown WWTF) which has some historic data collected after rain events (Figure B.1.5). Two water and two sediment samples will be collected quarterly from these five monitoring locations during the first year of sampling and analyzed for Enterococcus (Table A.7.1) resulting in 40 samples for each matrix.. Collected data will be evaluated and a subsequent sampling schedule and/or laboratory experiment(s) evaluating survival and growth in sediments will be developed, based on these results, in consultation with the TSSWCB PM and included in a revision or amendment to this QAPP.

d) Seepage samples

Ground water seeping into the creek may introduce enterococci to the creek or cause enterococci in sediments to re-suspend in the water column. Seepage meters will be used to collect two water samples at three of the five locations being sampled for water and sediments, in the upper Oso Creek/West Oso Creek watershed (Figure B.1.7 sites SW01, SW03, and SW04), following a procedure modified from Lee (1977). Sampling will follow rainfall and will be conducted for two events, resulting in twelve (12) samples. Details on procedure are described in Sampling Method Requirements.

Figure B.1.6. Approximate Locations of Groundwater Monitoring Wells.

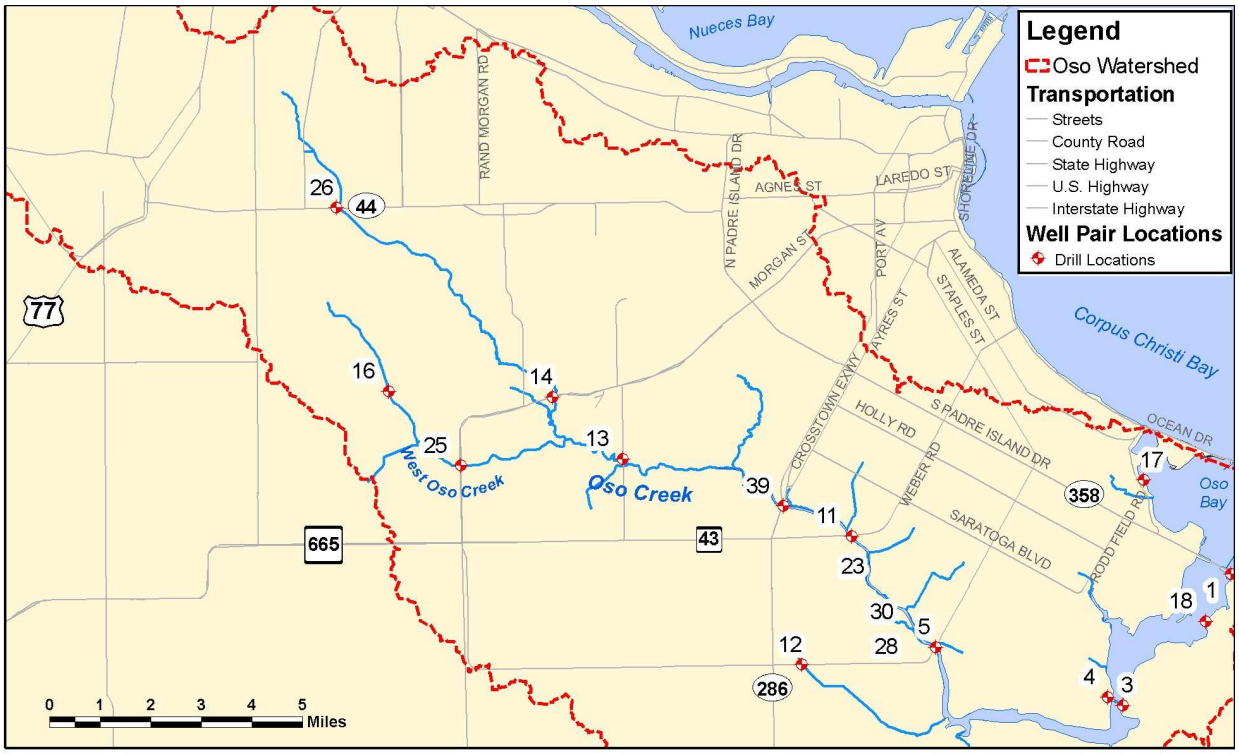

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Table B.1.2. Location of Groundwater Monitoring Wells.

Station_id	Description	site_id	Lon	Lat
GW 08A	Oso Creek at FM 763 nr 13029 (dn strm N bank) -Shallow	Site 13	-97.501900	27.711351
GW 08B	Oso Creek at FM 763 nr 13029 (dn strm N bank) -Deep	Site 13	-97.501900	27.711351
GW 09A	Oso Creek and FM 665 nr 18500 (Upstream West Bank) – Shallow	Site 14	-97.524254	27.729265
GW 09B	Oso Creek and FM 665 nr 18500 (Upstream West Bank) -Deep	Site 14	-97.524254	27.729265
GW 10A	West Oso Creek at Merritt Rd - USGS Site 1 -Shallow	Site 16	-97.576775	27.731150
GW 10B	West Oso Creek at Merritt Rd - USGS Site 1 -Deep	Site 16	-97.576775	27.731150
GW 14A	West Oso Creek at FM 665 S. bank d. strm nr 18501 -Shallow	Site 25	-97.553978	27.709423
GW 14B	West Oso Creek at FM 665 S. bank d. strm nr 18501 -Deep	Site 25	-97.553978	27.709423
GW 15A	Oso Creek at SH 44 nr 18499 (Upstream West Bank) -Shallow	Site 26	-97.593315	27.783936
GW 15B	Oso Creek at SH 44 nr 18499 (Upstream West Bank) -Deep	Site 26	-97.593315	27.783936

Figure B.1.7. Locations in Oso Creek Watershed at which Sediment and Water Samples will be Collected (marked as SW 01-05).

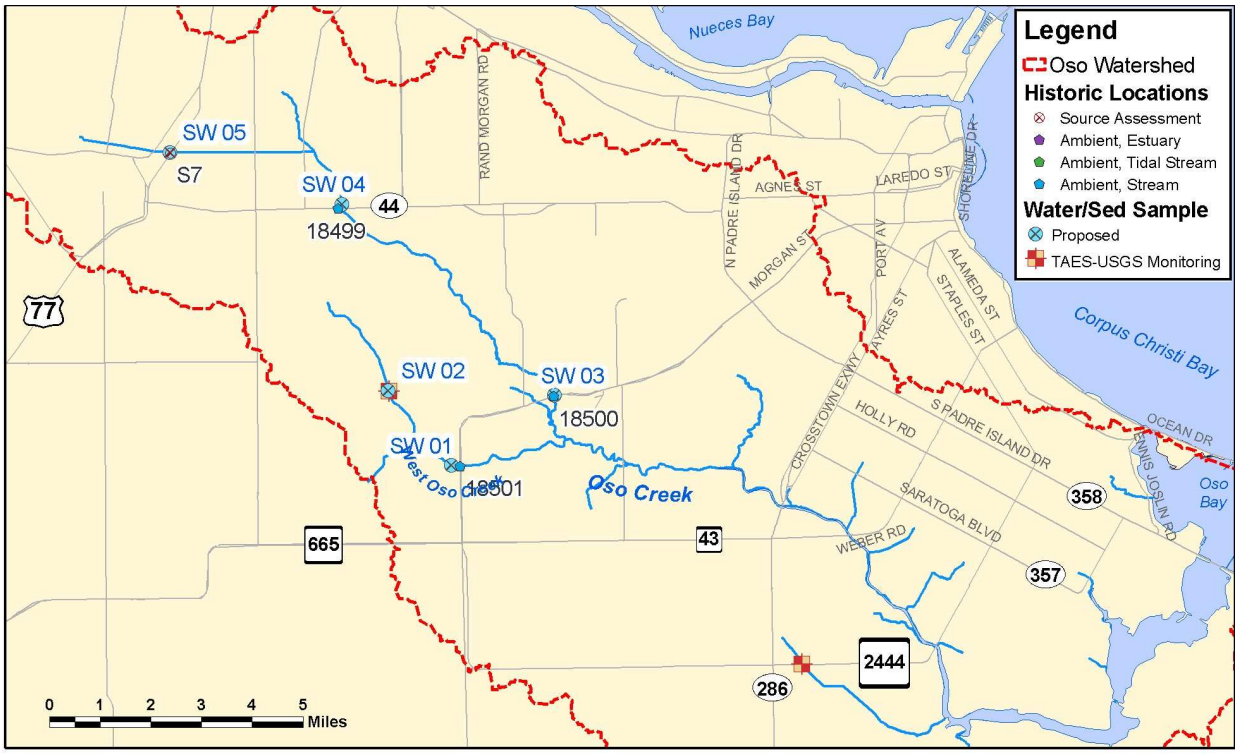

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Table B.1.3. Composite Sampling Regime for First Year.

Site	a) in-stream water	b) groundwater	c) upstream water & sediment	d) upstream seepage	TOTAL
18499	quarterly routine (8 samples)	quarterly routine shallow/deep (16 samples) rainfall-influenced shallow/deep (4 samples)	quarterly routine (8 samples)	rainfall-influenced (4 samples)	40
18501	quarterly routine (8 samples)	quarterly routine shallow/deep (16 samples) rainfall-influenced shallow/deep (4 samples)	quarterly routine (8 samples)	rainfall-influenced (4 samples)	40
18500	quarterly routine (8 samples)	quarterly routine shallow/deep (16 samples) rainfall-influenced shallow/deep (4 samples)	quarterly routine (8 samples)	rainfall-influenced (4 samples)	40
20198 (collocated w/ USGS 08211517)	-	quarterly routine shallow/deep (16 samples) rainfall-influenced shallow/deep (4 samples)	quarterly routine (8 samples)	-	28
20559	-	-	quarterly routine (8 samples)	-	8
13029 (collocated w/ USGS 08211520)	-	quarterly routine shallow/deep (16 samples) rainfall-influenced shallow/deep (4 samples)	-	-	20
13028	quarterly routine (4 samples) (through NRA CRP)	-	-	-	-*
TOTAL	24*	100	40	12	176*

* not including NRA CRP

e) Soil samples

A possible contribution of enterococci from soils has been suggested by results from another project (TSSWCB project 02-13; Dr. Fernandez, Texas AgriLife Research at Corpus Christi; USGS 2008), with fecal indicator analyses conducted by TAMU-CC (Mott). Extremely high concentrations of enterococci were found in runoff from agricultural fields in the watershed. Due to the complexity of factors involved in site selection for this sampling, results will only provide some indication as to whether additional studies are needed on this aspect. Soil sampling will focus primarily on surface soils (top 6 inches). Composite soil samples will be analyzed for Enterococcus. Selection of five soil sampling locations (fields) was made in consultation with the Nueces Soil and Water Conservation District #357, local USDA NRCS field staff, TSSWCB Regional Office personel, and Texas AgriLife Extension Service county agents. Additionally, the locations meet the following minimum criteria:

- The researchers must be granted open access to the field.
- The field must be located in the upper Oso Creek or West Oso Creek watershed.
- The field must be used primarily for agriculture.
- The field must be adjacent or drain directly to Oso Creek or West Oso Creek.

- The field should have areas of good drainage as well as low areas with poor drainage (i.e. field edge).
- Of the five locations (fields) there must be at least one each of sorghum, cotton, and grass (pasture) production.

Table B.1.4. Soil Sampling Sites.

NAME	Location (Soil types)	UTM14-E	UTM14-N	Crop Cover H1 2008
SO 01	W side CR 24 N. of FM 1889 (Orelia/Banqette/Victoria)	637435	3077620	Grain/Cotton
SO 02	W. side CR 24 S. of FM 1889 (Victoria/Orelia)	637072	3076280	Grain
SO 03	S. side SH 44 E. of CR 61 W. of Oso Creek (Victoria)	638880	3073450	Grain
SO 04	CR 30 N. side E. of CR 61 - (Victoria/Clareville)	640500	3068810	Grain/Wheat
SO 05	CR 57 W. side N. of CR 30- (Clareville/Victoria/Orelia)	640849	3069630	Cotton/Wheat
SO 06	CR 34 S. side E. of CR 61- (Orelia/Banqette)	639485	3070360	Grain
SO 07	FM 665 W. side N. of FM 43- (Victoria/Hidalgo/Clareville)	642182	3065770	Pasture
SO 08	S. side of CR34, W of FM892 (Victoria)	630808	3070673	Cotton
SO 09	N. side of SH357 between CR35 and CR37 (Victoria)	650405	3068271	Cotton

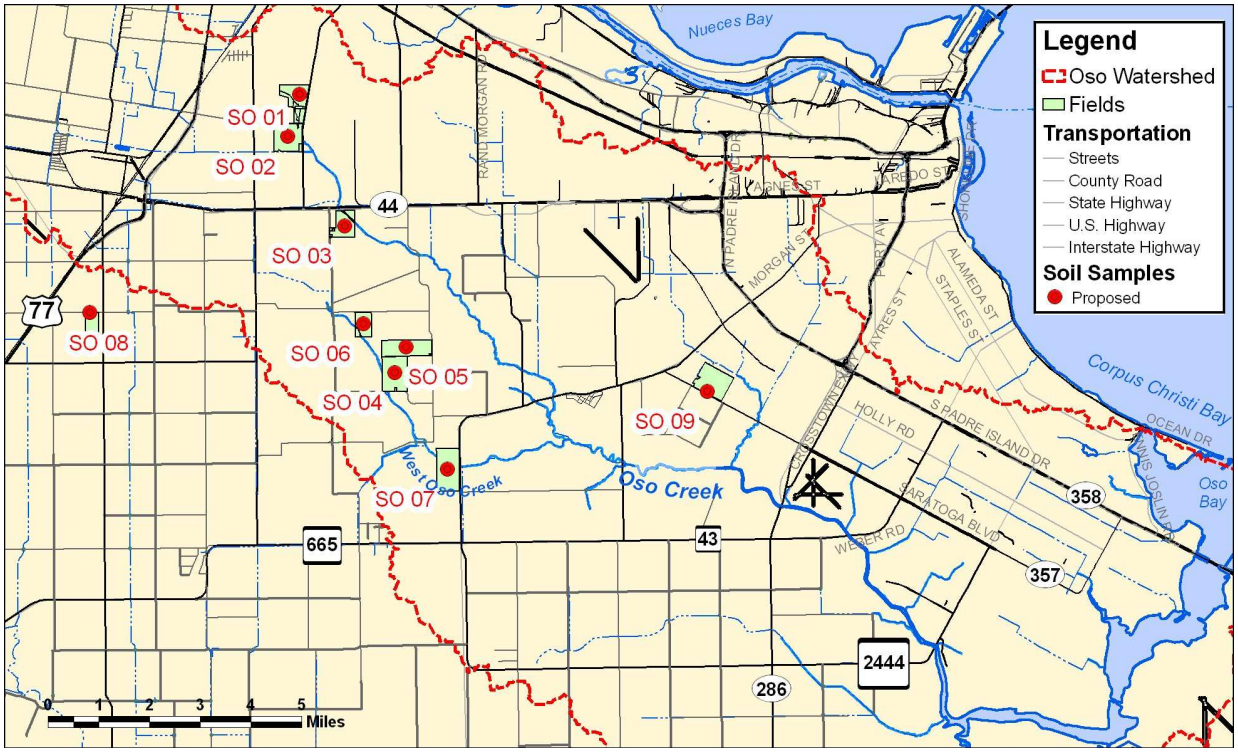


Figure B.1.8. Soil Sampling Sites.

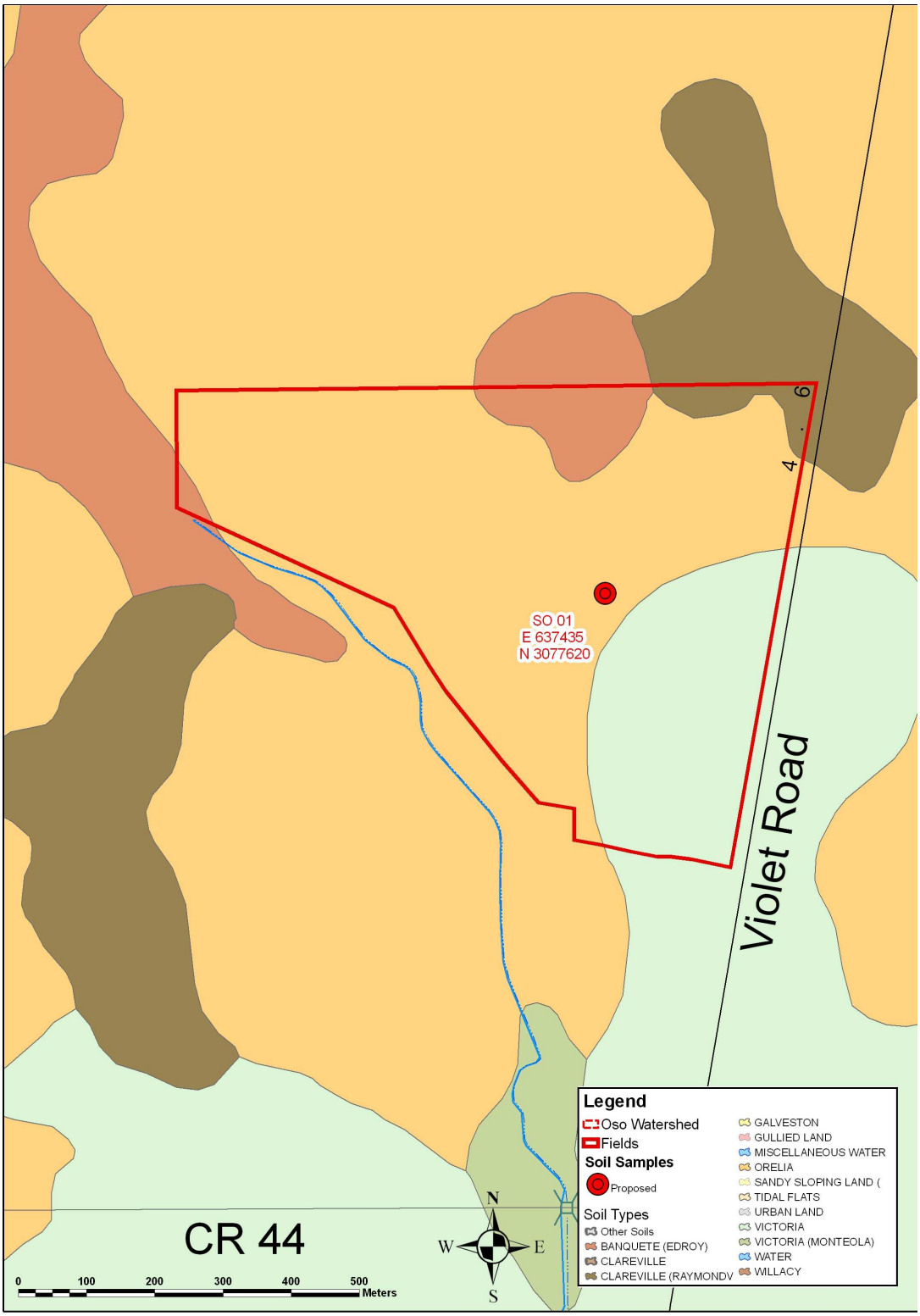


Figure B.1.9. Map showing location of Soil Sampling Site SO 01 and soil types.

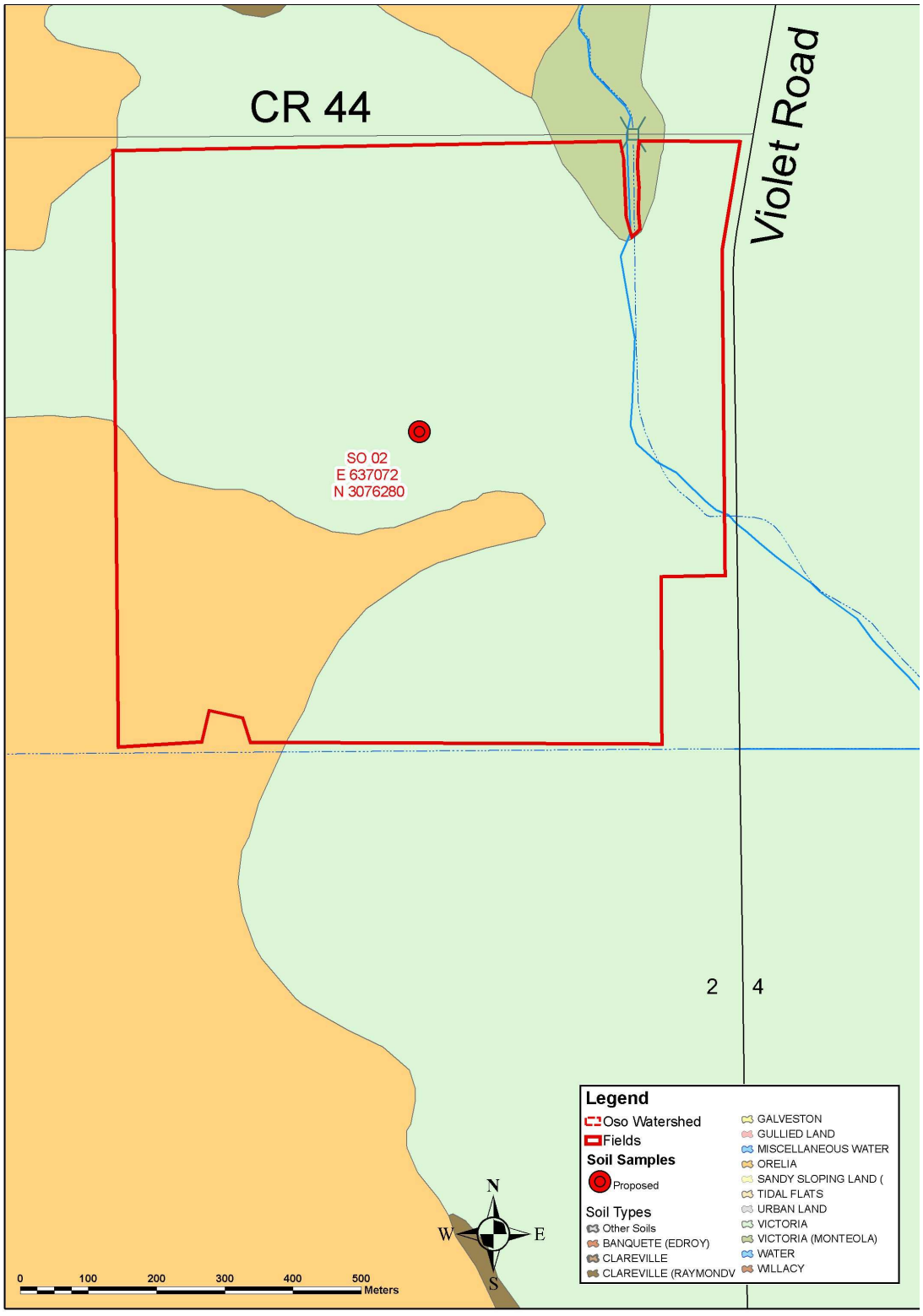


Figure B.1.10. Map showing location of Soil Sampling Site SO 02 and soil types.

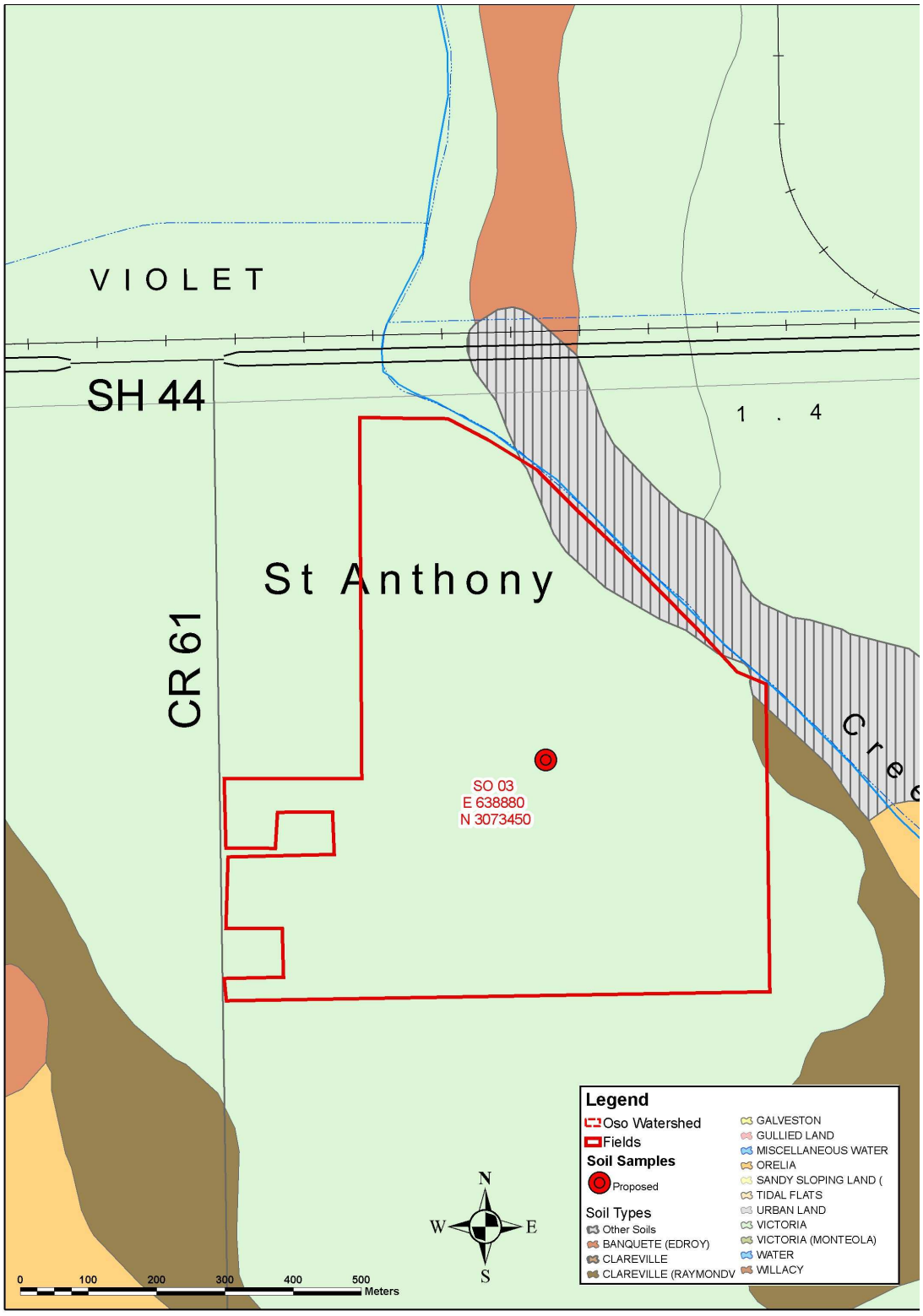


Figure B.1.11. Map showing location of Soil Sampling Site SO 03 and soil types.

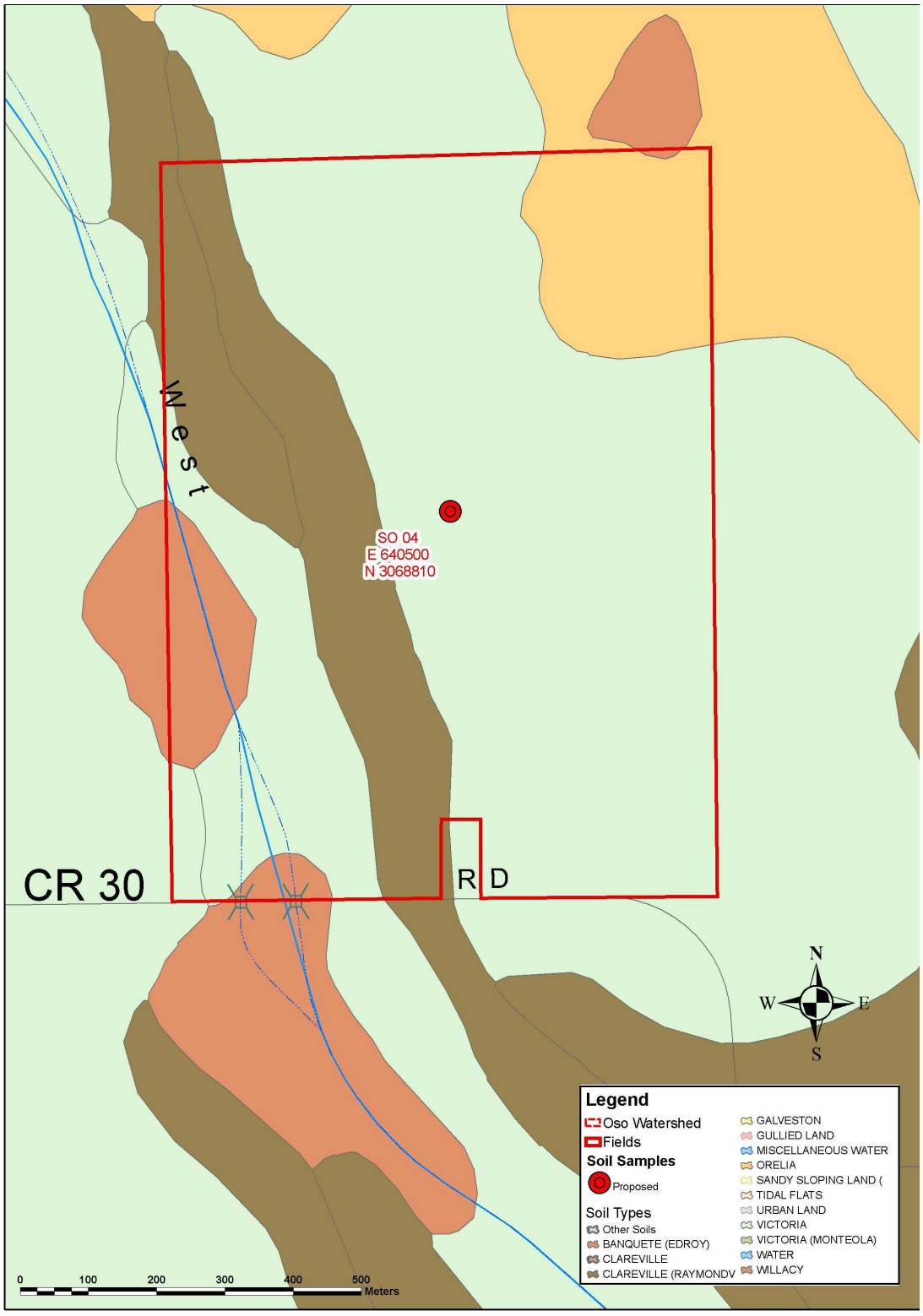


Figure B.1.12. Map showing location of Soil Sampling Site SO 04 and soil types.

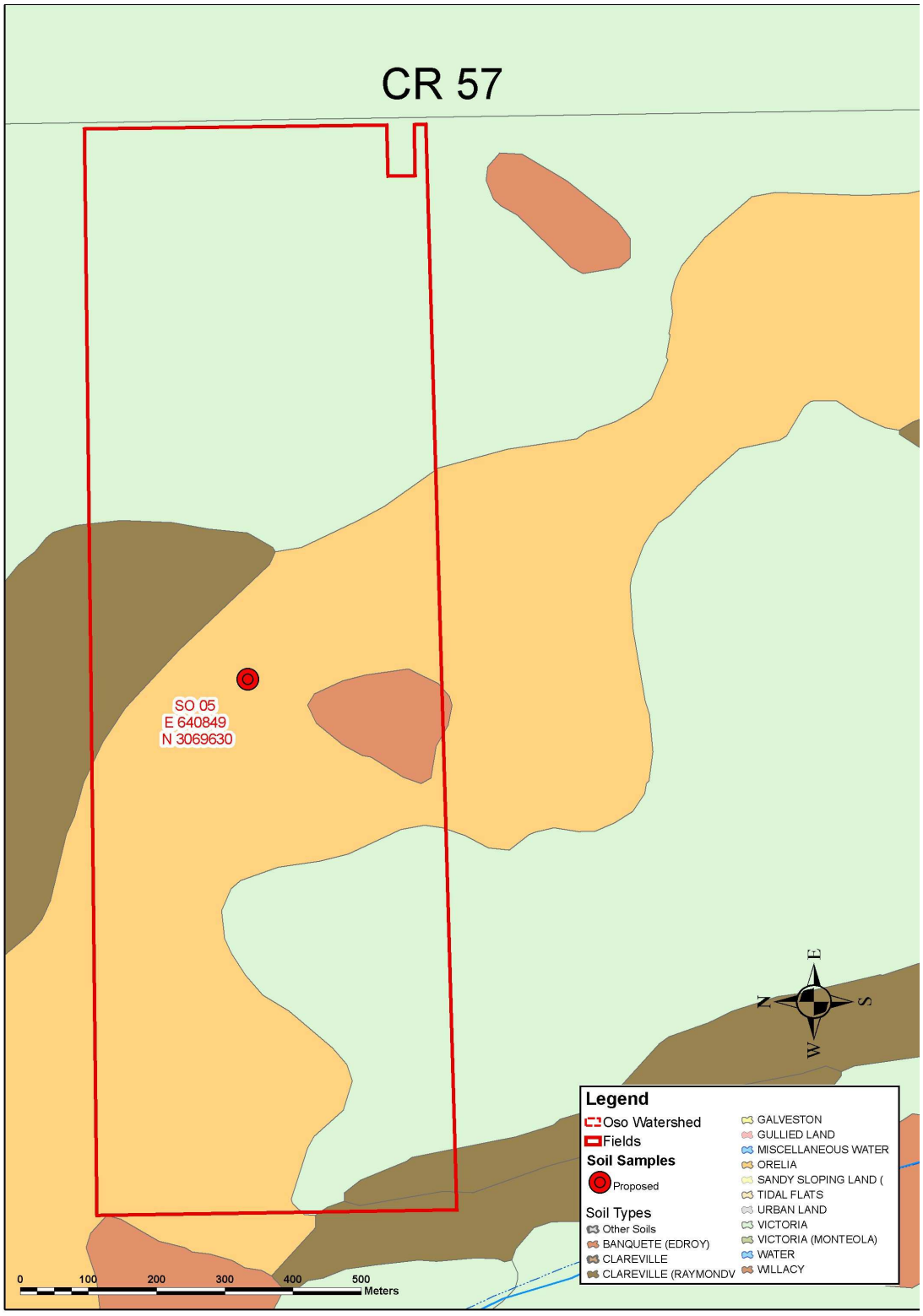


Figure B.1.13. Map showing location of Soil Sampling Site SO 05 and soil types.

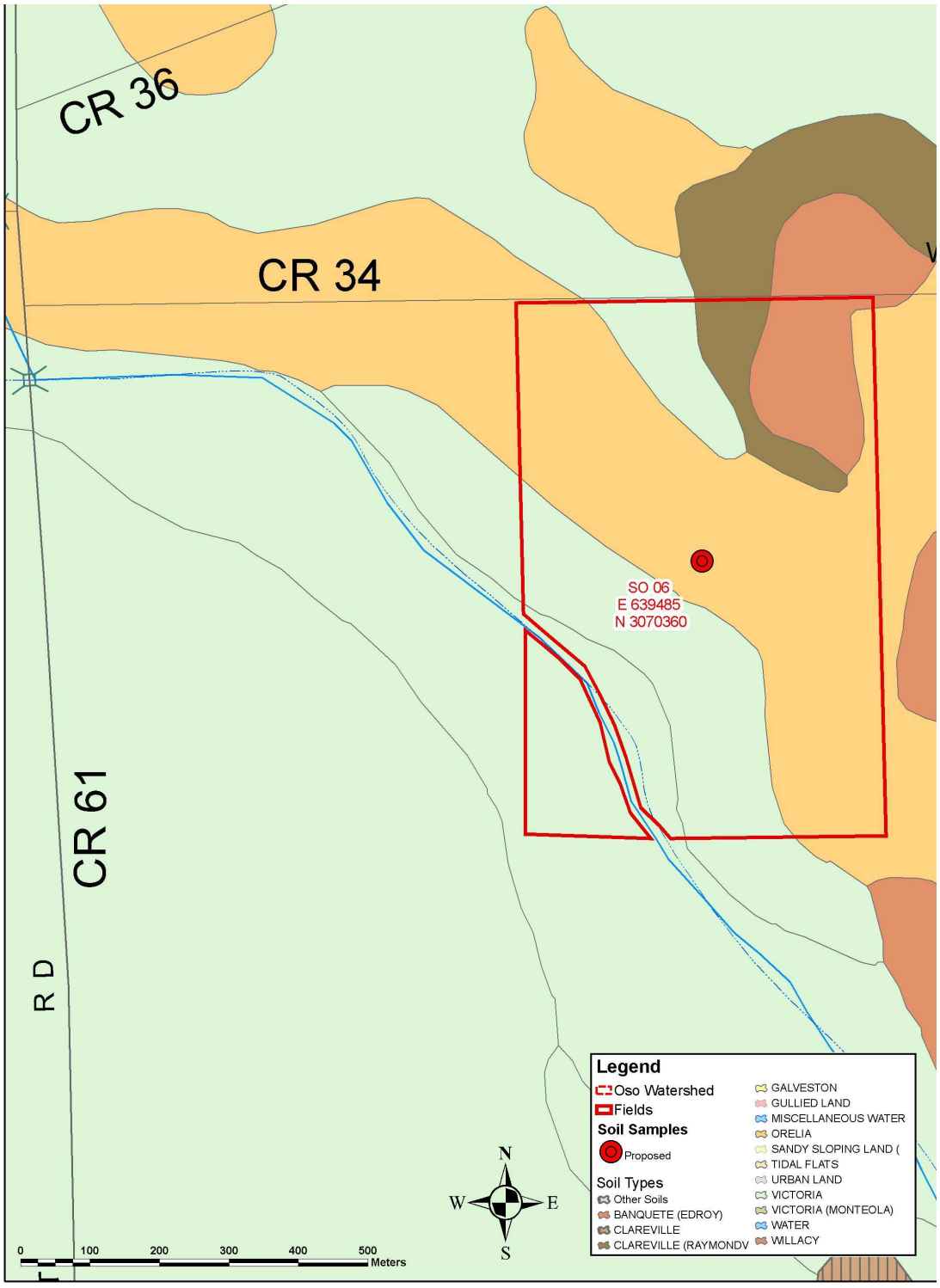


Figure B.1.14. Map showing location of Soil Sampling Site SO 06 and soil types.

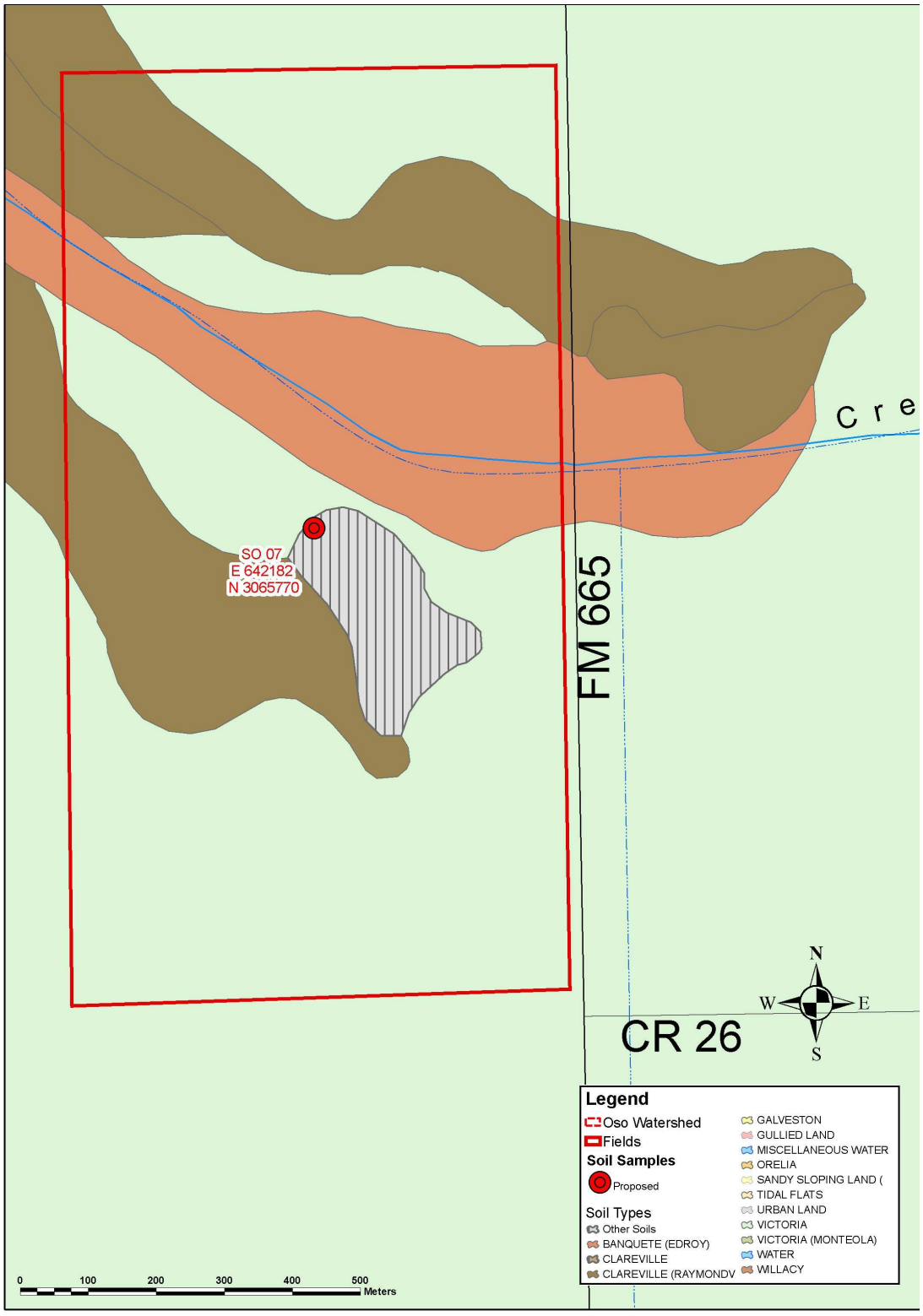


Figure B.1.15. Map showing location of Soil Sampling Site SO 07 and soil types.

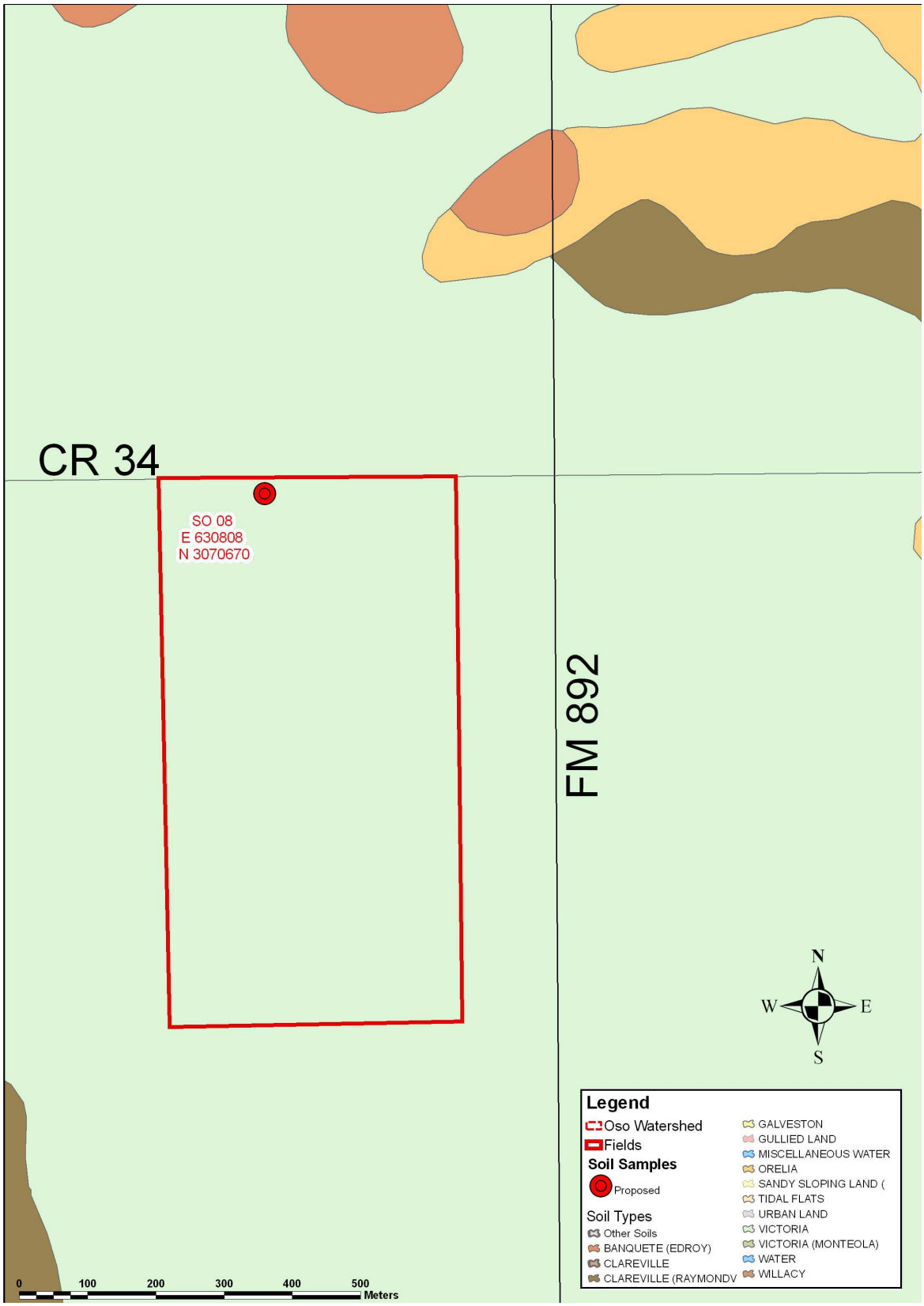


Figure B.1.16. Map showing location of Soil Sampling Site SO 08 and soil types.

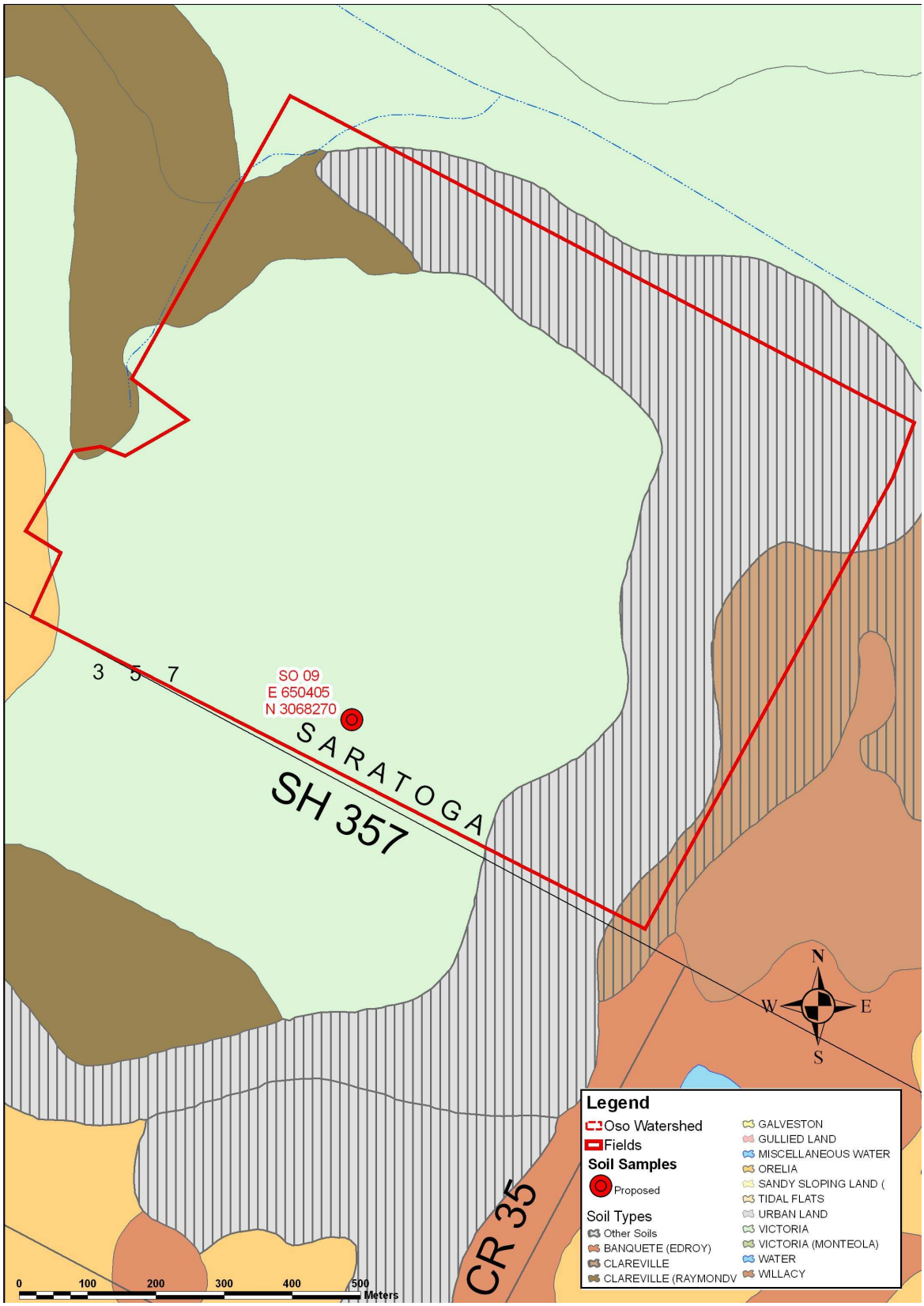


Figure B.1.17. Map showing location of Soil Sampling Site SO 09 and soil types.

First year sampling will consist of four (4) sampling events: two (2) wet and two (2) dry events. For each sampling event two (2) composite samples will be collected from each location: one from the well-drained portion of the field and one from the poorly drained portion of the field. Resulting in a total of 40 samples. Samples will be taken from both upland and low areas of the field. Composite samples from the top six inches will be used for analysis. Each composite sample will be composed of soil taken from up to five closely spaced (1-5 meters) sub-sites.

Small scale studies of survival and re-growth in sediments and/or agricultural soil will be conducted dependent on the initial sampling results. The design of these experiments will be developed following the first year of sampling. All methods utilizized will follow QAPP requirements outlined in this document.

Bacterial Source Tracking (BST)

Animal sources of enterococci in Oso Creek will be determined using two library-dependent BST techniques – Carbon Source Utilization (CSU) profiling and Antibiotic Resistance Profiling (ARP) of isolates. Additionally a subset of ~100 isolates will be analyzed for *esp* gene detection (library-independent BST) to provide added confidence in the data..

For library development fecal samples from appropriate animal sources will be collected by TAMU-CC personnel, under the supervision of the PLs. Land use and sanitary survey information collated by the PLs under an approved QAPP for a previous TCEQ project for Oso Creek/Oso Bay (2005) and comments from multiple Oso Creek/Oso Bay TMDL Stakeholder meetings including one held January 28, 2008 with the TSSWCB (detailed in April 2008 QPR) will be used to determine potential source animals and appropriate locations for fecal collection.

Land use information was obtained from the 1992 National Land Cover Dataset (Table B.1.5), and permitted discharge information was taken from the TCEQ permit database (Table B.1.6). The Oso Bay/Creek watershed was assessed using aerial maps to examine land use and accessibility for sampling. Livestock, colonias and any other potential fecal sources (e.g. landfill) were observed, recorded and marked on a map (Figure A.4.1). Field surveys were conducted in 2005 by the TAMU-CC PLs, the TAMU-CC QAO and the Lab Manager and Field Supervisor. GPS coordinates of each potential site were taken by one of the PLs (Richard Hay). Follow-up surveys have been initiated in 2008 to confirm locations of animals such as livestock.

Table B.1.5. Land use in the Oso Bay/Oso Creek Watershed (Hay and Mott, 2005).

Land Use Types	Percent
Planted/Cultivated	67.8
Urban Development	13.8
Grasslands	5.2
Water	4.5
Shrubland	3.8
Wetlands	2.8
Forested Upland	2.0
Barren	0.2

Source – USEPA/USGS 1992 National Land Cover Dataset.

Table B.1.6. Permitted Dischargers in the Oso Bay/Oso Creek Watershed (Hay and Mott, 2005).

Permitted Discharger	TCEQ Permit No.	Permitted Daily Avg. Flow (MGD)
American Electric and Power Barney Davis Power Station (1)	01490-000	540
City of Corpus Christi Oso WWTP (2)	10401-004	16.2
City of Corpus Christi Greenwood WWTP (3)	10401-003	8.0
Texas A&M University CBI La Coss Facility (4)	03646-000	5.04
City of Robstown (5)	10261-001	2.4
Equistar Chemical LP Corpus Christi Plant (6)	02075-003	2.0
Tennessee Pipeline Construction Co. Cuddihy Airfield WWTP (7)	14228-001	0.06
Corpus Christi Peoples Baptist Church Roloff WWTP (8)	11134-001	0.02
Texas A&M University Agriculture Research Ext. (9)	11345-001	0.0015
City of Corpus Christi Storm Water (10)	04200-000	NA

Figure B.1.18. Map Developed from Sanitary Survey, showing Potential Sources of Bacteria Loading into Oso Bay/Oso Creek.

Map Document: (K:\TSSWCB\maps\BacteriaSources_4x6.mxd)
6/19/2008 -- 1:49:07 PM

The TAMU-CC library currently has ~400 Enterococcus isolates from four sources: human, cow, seagull and dog collected in the Coastal Bend area. These isolates have CSU profiles, but will need to be re-grown for ARP analysis. The library will be expanded to total at least 1,000 enterococcus isolates with both CSU and ARP profiles. Potential sources include human/sewage (malfunctioning septic systems, WWTFs overloaded after rainfall, unpermitted discharges), livestock (cattle, horses, goats, turkeys/chickens), domestic pets (dog, cat), birds (avian wildlife), wildlife (non-avian) e.g. coyote, fox, opossum, feral hog, raccoons, bats, field rats/mice. Specific numbers of isolates for each source will be determined after re-evaluation of the existing library based on original collections and location of samples collected. Specific locations will be determined and documented on field data sheets during surveys of the area.

Unknown source isolates will be analyzed from water and sediment samples collected by TAMU-CC personnel. The goal is to analyze ~800 unknown source isolates (average 25-50 isolates/sample) to determine animal sources of enterococci in the upper sections of Oso Creek. The plan is to analyze ~600 isolates from the three historic stations (total 200 per station) by collecting 50 isolates per station from four seasonal sampling events (three ambient dry and one following rainfall). The additional 200 isolates, 50 per source will be collected from wells, soil, sediment, subsurface, with locations and frequency of sampling dependent on findings from the monitoring and sediment sampling conducted in Year 1 and after discussion with TSSWCB

personnel. This will be included in QPRs. Methods of analysis will all follow those detailed in the QAPP for isolation of enterococci from water, sediments and fecal samples and CSU and ARP protocols. For *esp* detection, samples will be collected from the three historic stations under ambient conditions and following rainfall . Again, some samples may be from groundwater or subsurface water, dependent on initial monitoring results.

The waterborne constituents that will be measured are shown in Table B.1.7

Table B.1.7. Waterborne Constituents.

Parameter	Status	Reporting Units
Laboratory Parameters		
Enterococcus	Critical	colonies per 100 milliliters (col/100ml)
Enterococcus ARP	Critical	Zone diameters (mm)
Enterococcus CSU	Critical	Species and well intensity (NA)
Esp gene	Critical	Presence/Absence
Field Parameters		
Dissolved Oxygen	Non-critical	milligrams per liter (mg/L)
Potential Hydrogen (pH)	Non-critical	pH standard units
Specific Conductance	Non-critical	microsiemens per centimeter (µS/cm)
Water Temperature	Non-critical	degrees Celsius (°C)
Salinity	Non-critical	parts per thoursand (ppt)
Flow Rate	Non-critical	meters per second (m/s); cubic liters per second (cls)
Flow Severity	Non-critical	1-no flow, 2-low, 3-normal, 4-flood, 5-high, 6-dry
Water depth at sample	Non-critical	centimeters (cm)

Section B.2 Sampling Method Requirements

TAMU-CC will follow the field sampling procedures documented in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008) for collection of water samples and measurement of field parameters. Field parameters will be measured with a YSI or Hydrolab $H_20^{®}$ water quality multiprobe instrument. Additional procedures for field sampling outlined in this section reflect specific requirements for sampling under this project and/or provide additional clarification.

All water sampling will follow procedures described in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). In-stream water samples will be collected when possible by wading into the stream to reach the center of the flow. If the center of the flow cannot be reached, then samples will be collected from a bridge using a bucket. For stations where no bridge is nearby a sample will be collected from the shoreline and this will be noted in the field log book. In cases of high flow (after rainfall) safety will be the overall concern and the professional judgement of the field supervisor will be used in determining sampling procedures. Samples will be collected from depths specified in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008) for waters of different depths. Probe parameters will be measured in situ when possible, but when this is not possible or unsafe, the protocol for field measurements from a bucket will be used as detailed in *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). Flow measurements will be taken first when possible, to delay collection of bacteria and water samples that have limited holding times. Should flow measurements be taken first, multiprobe instruments will not be deployed in the same area and water sample collections will be taken in an undisturbed area. Flow measurement methods and flow estimation procedures will follow guidelines stated in *TCEQ Surface Water Quality Monitoring Procedures, Volume I* (October 2008). Flow measurements will be collected mechanically. Two exceptions are: no flow at a stream site and only isolated pools remain in the stream bed, and dry stream bed containing no water.

Ground water sampling for Enterococcus will follow standard procedure as set forth in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Chemical Monitoring Methods for Water, Sediment, and Tissue* (October 2008) except the sample will be drawn from the well in the following manner: prior to groundwater sample collection, at least three casing volumes of water must be removed from the well using a new disposable bailer, or a dedicated bailer (a bailer that is assigned for use only in a specific well) or peristaltic pump using new tubing to ensure that the sample is from the aquifer and not stagnant well water. The casing volume is the length of the water column times the well diameter.

Equation 1. Casing volume calculation.
Depth x (½ x diameter)^2 x PI x 1000 = casing volume

Where:
Depth (meters) = Total Depth of Well from Measurement point – Depth to water from measurement point.
Diameter (meters) = Inside width of the casing
PI = 3.14
Casing volume (liters)

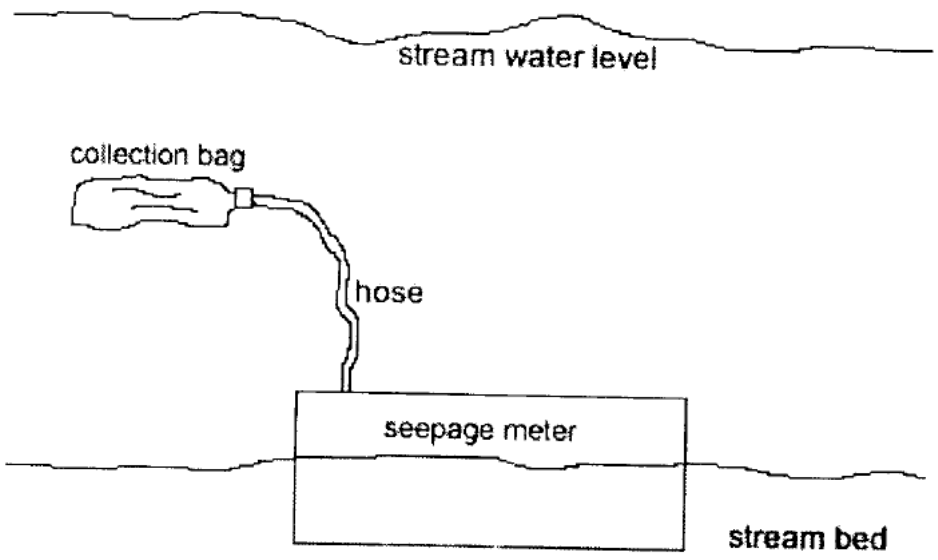
EXAMPLE: Casing volume purge calculation for a 2 in diameter well

1. 2 in diam well = 0.05 meters
2. Depth to water = 3.05 meters (10ft)
3. Well depth = 6.1 meters (20ft)
4. Water column = 3.05 meters
5. Casing volume = 3.05 meters x (.5 x .05meters)^2 x PI x 1000 = 6.17 liters
6. Purge volume = 6.17 liters x 3 = 18.51 liters= 4.9 gallons

In the event that the well is dry prior to removing three casing volumes of fluid, then the well will be allowed to recover and sampling will take place when there is sufficient fluid to meet the required sampling volume, or make a measurement.

Seepage sampling will be conducted using seepage meters to collect water samples. Methods for installation and collection of water samples using a seepage meter will be modified from Lee (1977). Seepage meters will be built from the bottom 15 cm of a 5-gallon plastic bucket, with 50-150 cm tubing (depending on water depth) and a collapsible wall triple laminate bag, or latex balloon for sample collection. (Figure B.2.1). The meters will be located in a relatively soft area in the stream bed and pushed into the bed by hand. The tubing is attached and filled with ambient water and a collapsible collection bag containing 100 ml of deionized water will then be attached. The time when the bag is attached will be recorded on a field data sheet. After at least 24 hours, and up to 3-4 days, later the collection bag will be removed, capped and transported to the lab for analysis.

Figure B.2.1. Seepage Meter Configuration.



Soil and sediment sampling will follow procedures described in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). Field data sheets will be used to document field parameters. Sediment samples will be collected from the shoreline when the sediment is covered with water. In all cases safety will be the overall concern and the professional judgement of the field supervisor will be used in determining sampling procedures. Sediment samples will be collected using sterile 6 inch pvc corers or for deeper samples using a coring device with a removable sleeve. Soil samples will be collected following the Soil Science Society of America *Methods of Soil Analysis Part 2 – Microbiological and Biochemical Properties* (1994). Composite samples from the top six inches of soil from up to five closely spaced (1-5 meters) sub-sites will be collected. Three subsamples will be taken from each composite and transferred to sterile whirl-pak bags for transport to the laboratory.

Fecal sampling procedures will depend on the source of the material. Human/sewage samples will be collected from WWTFs, and septic system pump-out material if available. Livestock and pet samples will be collected with appropriate permission from owners, or from veterinary clinics, provided animals reside within the watershed and antibiotics have not been administered to the animal. Bird samples (avian wildlife) will be collected only from visually observed depositions. Wildlife samples (avian and non-avian) will be obtained in collaboration with Texas Parks and Wildlife Department personnel using their approved methods, from fecal samples from areas where animals were visually observed defecating, or from gut samples collected from

animals recently killed by cars (within 24 hours) or legally harvested by hunters who have agreed to work with TAMU-CC. Gut samples will be collected by using sterile swabs inserted anally or by cutting into the intestine using a sterile scalpel. In all cases samples will not be collected unless there is a high degree of certainty of the source of the fecal material. Samples will be collected from within the fecal material, using a self-contained sterile swab. Human samples will be collected directly from untreated sewage and outflows using sterile specimen containers. The swabs and containers will be held in sterile biohazard bags and placed on ice until delivery to the lab. SOPs are available at TAMU-CC.

Sample Volume, Container Types, Minimum Sample Volume, Preservation Requirements, and Holding Time Requirements.

Table B.2.1. Container Types, Preservation Requirements, Temperature, Sample Size, and Holding Time Requirements.

Parameter	Matrix	Container	Preservation	Sample Volume	Holding Time
Enterococcus	Water	One liter sterile polypropylene bottle	4°C, sodium thiosulfate if residual chlorine is present[2]	500-750 ml	6 hrs, plus 2 lab hrs[1]
Enterococcus	Soil/sedi-ment	Sterile corers or whirlpak bags	4°C	50-100 g or content of corer/bag	6 hrs, plus 2 lab hrs[1]
Fecal specimen	Feces	polypropylene, screw cap, sterile specimen containers or using BD BBL EZ Culture swabs	4°C	~30 g	5 days

[1]Six hours to deliver to laboratory. In the case that this 6-hour holding time is not met, the Enterococcus quantitative count will be flagged, though the non-quantitative source identification (BST) will still be valid.
[2]Each sample will be tested for presence of residual chlorine using a potassium iodide test strip; results will be documented on the field data sheet.

Sample Containers

Water samples for bacteria analysis will be collected in labeled one liter screw-cap polypropylene bottles which are cleaned and autoclaved prior to each use. The following TAMU-CC SOP contains the specific steps used for container cleaning and is available for review upon request: *Preparation of Sterile Supplies* (includes sampling bottles and equipment used in field operations). Sediment and soil samples will be collected using sterilized corers and then held/transported in sterile whirlpak bags. Fecal samples will be collected in pre-packaged sterile, polypropylene, screw cap specimen containers or using pre-packaged sterile BD BBL EZ Culture swabs. Samples will be transported and analyzed under the same conditions (B3). After isolation of Enterococci from the fecal samples, specimen containers and swabs will be placed in biohazard bags and autoclaved prior to disposal.

Safety is an issue when working with raw sewage samples due to the bacterial concentration and possible hazards to humans. Hazardous material safety handling instructions will be included in a file for driver to carry that will be visible on seat or dash of vehicle in case of accident or being stopped by the highway patrol. Biohazard signs will be placed on the cooler containing raw sewage samples collected for transport to the TAMU-CC laboratory.

Processes to Prevent Cross Contamination

The *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008) outlines the necessary steps to prevent cross-contamination of samples. These will include direct collection into sample containers, when possible. Field QC samples as discussed in Section B5 are collected to verify that cross-contamination has not occurred. In cases *where a bucket is used to collect water samples from a bridge all steps outlined in the TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008) will be followed to prevent cross-contamination.

Documentation of Field Sampling Activities

Field sampling activities are documented on field data sheets as presented in Appendix B. Flow work sheets, multi-probe calibration records, and records of bacteria analyses are part of the field data record. For all visits, station ID (if applicable), location, sampling time, sampling date, sampling depth, preservatives added to samples and sample collector's name/signature are recorded. Values for all measured field parameters are also recorded. Detailed observational data are recorded including as appropriate: water appearance, weather, biological activity, stream uses, unusual odors, specific sample information, missing parameters (items that were to have been sampled that day, but were not), days since last significant rainfall, and flow severity.

Recording Data

For the purposes of this section and subsequent sections, all field and laboratory personnel follow the basic rules for recording information as documented below:

1. Legible writing in indelible, waterproof ink with no modifications, write-overs or cross-outs;
2. Correction of errors with a single line followed by an initial and date;
3. Close-outs on incomplete pages with an initialed and dated diagonal line.

Deficiencies, Nonconformances and Corrective Action Related to Sampling Requirements

Deficiencies are defined as unauthorized deviations from procedures documented in the QAPP. Nonconformances are deficiencies that affect quality and render data unacceptable or indeterminate. Deficiencies related to sampling method requirements include, but are not limited

to, such things as sample container, volume, and preservation variations, improper/inadequate storage temperature, holding-time exceedances, and sample site adjustments.

Deficiencies are documented in logbooks and field data sheets by field or laboratory staff and reported to the field/laboratory supervisor. The supervisor will notify the TAMU-CC QAO. If the situation requires an immediate decision concerning data quality or quantity, a TAMU-CC PL will be notified within 24 hours. The TAMU-CC PL will notify the TAMU-CC QAO of the potential nonconformance. The TAMU-CC QAO will record and track the CAR to document the deficiency.

The TAMU-CC QAO, in consultation as appropriate with the TAMU-CC PLs, will determine if the deficiency constitutes a nonconformance based on best professional judgment.. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TAMU-CC PLs in consultation with TAMU-CC QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TAMU-CC QAO.

CARs document: root cause(s); programmatic impact(s); specific corrective action(s) to address any deviations; action(s) to prevent recurrence; individual(s) responsible for each action; the timetable for completion of each action; and the means by which completion of each corrective action will be documented.. The TSSWCB will be notified of excursions that affect data quality with QPRs. All CARs will be submitted with QPRs. In addition, significant conditions (i.e., situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported to the TSSWCB immediately.

Section B.3 Sample Handling and Custody Requirements

Chain-of-Custody

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. A sample is in custody if it is in actual physical possession or in a secured area that is restricted to authorized personnel. The COC form is used to document sample handling during transfer from the field to the laboratory. The sample number, location, date, changes in possession and other pertinent data will be recorded in indelible ink on the COC. The sample collector will sign the COC and transport it with the sample to the laboratory. At the laboratory, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. Sample numbers will then be recorded into a laboratory sample log, where the laboratory staff member who receives the sample will sign it. The list of items below are included on the COC form (See Appendix C for sample form).

1. Date and time of sample collection, shipping and receiving
2. Site identification
3. Sample matrix
4. Number of containers
5. Preservative used or if the sample was filtered
6. Analyses required
7. Name of collector
8. Custody transfer signatures and dates and time of transfer
9. Name of laboratory admitting the sample
10. Bill of lading (if applicable)

Sample Labeling

Samples will be labeled on the container with an indelible, waterproof marker. Label information will include site identification, date, sampler's initials, and time of sampling. A COC form will accompany all sets of sample containers.

Sample Handling

Samples will be transported in ice chests at 4 ^{o}C to the TAMU-CC laboratory for analysis within the required holding time. A standard COC form will be filled out with collector signature (Appendix C) to include field parameters and date/time collected. On arrival at the laboratory the TAMU-CC Laboratory Supervisor or trained analyst will inventory the samples against the accompanying COC. Any discrepancies will be noted at that time, remediated if possible, and the COC will be signed for acceptance of custody. Times of collection will be checked to ensure holding times have not been exceeded and any exceedance will be documented and reported to

the QA Officer. In the case of water samples an additional bottle of the same specifications as the sample bottles, containing the same volume of water is included in the ice chest and will be used to check temperature of the samples on arrival in the laboratory. Any exceedances in holding times or temperature violations will be documented and reported to a TAMU-CC P.I..

Upon receipt of samples, laboratory IDs are assigned and samples are checked for preservation (as allowed by the specific analytical procedure). Samples are then filtered or otherwise analyzed. In cases where immediate analysis is not required, samples will be pretreated as necessary and placed in a refrigerated cooler dedicated to sample storage.

Deficiencies, Nonconformances and Corrective Action Related to Chain-of-Custody

Deficiencies are defined as unauthorized deviations from procedures documented in the QAPP. Nonconformances are deficiencies that affect quality and render data unacceptable or indeterminate. Deficiencies related to COC include, but are not limited to, delays in transfer, resulting in holding time violations; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc.

Deficiencies are documented in logbooks and field data sheets by field or laboratory staff and reported to the field/laboratory supervisor. The supervisor will notify the TAMU-CC QAO. If the situation requires an immediate decision concerning data quality or quantity, a TAMU-CC PL will be notified within 24 hours. The TAMU-CC PL will notify the TAMU-CC QAO of the potential nonconformance. The TAMU-CC QAO will record and track the CAR to document the deficiency.

The TAMU-CC QAO, in consultation as appropriate with the TAMU-CC PLs, will determine if the deficiency constitutes a nonconformance based on best professional judgment. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TAMU-CC PLs in consultation with TAMU-CC QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TAMU-CC QAO.

CARs document: root cause(s); programmatic impact(s); specific corrective action(s) to address the deficiency; action(s) prevent recurrence; individual(s) responsible for each action; the timetable for completion of each action; and, the means by which completion of each corrective action will be documented. The TSSWCB will be notified of excursions that affect data quality with QPRs. All CARs will be submitted with QPRs. In addition, significant conditions (i.e., situations that, if uncorrected, could have a serious effect on safety or validity or integrity of data) will be reported to TSSWCB immediately.

Section B.4 Analytical Methods Requirements

The analytical methods are listed in Table B.4.1 of Section B.4.

Dissolved oxygen, water temperature, conductivity and pH of water at sampling sites for this project will be measured in-situ using YSI or Hydrolab multiprobe field sampling equipment.

The remainder of the parameters will be analyzed by the Environmental Microbiology Laboratory at TAMU-CC.

Laboratories collecting data under this QAPP are, at a minimum, compliant with ISO/IEC Guide 25 and the TAMU-CC EML has interim NELAC accreditation. Standard operating procedures have been established for all procedures undertaken by TAMU-CC staff that concern water quality monitoring and analysis, and copies of these SOPs are available for review by the TSSWCB. BST SOPs are included in Appendix D.

Procedures for laboratory analysis will be in accordance with the most recently published edition of Standard Methods for the Examination of Water and Wastewater, the latest version of the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008), 40 CFR 136, or other reliable procedures acceptable to TSSWCB. Exceptions to this include analyses and sample matrices for which no regulated methods exist (i.e., BST).

In this project, enterococci in water samples will be enumerated using EPA Method 1600: Membrane Filter Test Method for Enterococcus in Water USEPA (1997, 2000) (http://epa.gov/waterscience/methods/biological/1600enterococcus.pdf)

Sediment and soil samples will be pre-treated either by preparing a dilution series followed by plating or by shaking with dispersant and filtration onto the same medium and counting as described in EPA Method 1600.

Enterococci will be isolated from fecal samples following the TAMU-CC SOP available on request. Fecal swabs will be used to inoculate mEnterococcus plates (Difco) and incubated for 48 h at 41°C. The plates will be streaked for isolation following standard microbiological techniques. Isolates will be transferred as needed to obtain pure cultures. At least two pure isolates with a maroon hue will be transferred from each plate to Difco Tryptic Soy Agar (Becton Dickinson and Co.) slants for storage. Identifications will be confirmed, isolates speciated and carbon source utilization profiles determined using a standard rapid test system - Microlog™ Microbial Identification System (Biolog, Inc., 3938 Trust Way, Hayward, CA, 94545). Isolates will be grown on Biolog Universal Growth (BUG) plates with 5% Sheep's Blood at 35^{o}C for 24h. Suspensions of each isolate will be made in Inoculating Fluid, based on a turbidity of 20% T± 2%, and used to inoculate GP2 MicroPlates™. After incubation at 35^{o}C for 24h, plates will be read using a Biolog MicroStation™ Reader to obtain well color intensity data, and manually

for positive/negative (+/-) carbon utilization in each well. Both color intensity and +/- readings will be transferred to SPSS® spreadsheets for statistical analyses using discriminant analysis. Biolog™ printouts include either well color intensity or positive/negative data. Each printout also includes the identification of the isolate to genus or species. Dependent on the proportion of isolates identified to genus but not to species by the Biolog Microstation™ Reader, those isolates not speciated may be swabbed onto mEI Agar plates and incubated at 41°C for 24h. Isolates whose colonies exhibit a blue halo on mEI Agar (i.e. that would be identified as enterococci by EPA Method 1600) may be included in the database. Isolates will be stored permanently in a -80°C freezer.

Water sample enterococci isolates (approximately 25 per sample) will be obtained following EPA Method 1600 as described in "Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and Escherichia coli" (2000) EPA/821/R-97/004 and following incubation procedures and quality control methods outlined in *Standard Methods for the Examination of Water and Wastewater*, 21st ed., 2005. Every effort will be made to isolate the required number of isolates. However, it should be noted that in some instances the bacteria may not be present in sufficient concentrations to achieve this objective. In such cases, the volume filtered and the number of isolates obtained will be recorded and analyses will proceed using those isolates that can be obtained. Colonies will be transferred to mEnterococcus plates to obtain pure cultures. Procedures will then follow those described above for fecal isolates using the standard rapid test system - Micrologᵀᴹ Microbial Identification System (Biolog, Inc.). Cultures will be maintained on tryptic soy agar (TSA) slants. Isolates will be stored permanently in a - 80°C freezer.

Antibiotic resistance profiles will be determined for each Enterococcus isolate. The analytical procedures for antibiotic resistance profiling will follow the standardized Kirby Bauer Disk Diffusion method with a panel of antibiotics following the standard method of the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2006, 2008). Zones of inhibition will be measured using an automated image analyzer to ensure uniformity for future comparisons with Enterococcus isolates from unknown sources as detailed in the TAMU-CC SOP (available on request) following NCCLS (2006) as approved in a previous QAPP (2003) "Development of an E. coli bacterial source tracking library and assessment of bacterial sources impacting Lake Waco and Lake Belton" (TSSWCB project 02-10). An additional antibiotic, vancomycin will be included in the panel as appropriate for a Gram positive bacterium. Standard ATCC bacteria strains, for which acceptable zone diameters to certain antibiotics have been determined by CLSI will be included with each analytical batch to ensure consistency and to validate results, following published clinical standards as described in Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard-Ninth Edition. CLSI document M2-A9 (2006), CLSI. 2008; Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Third Edition. CLSI document M31-A3 and CLSI. 2008. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S18 (2008).

Approximately 100 water samples will be analyzed for the presence of the *esp* gene following the TAMU-CC/Harwood protocol (SOP) described in Appendix D which is based on the paper by McQuaig, S.M., Scott, T.M., Harwood, V.J., Farrah, S.R. and Lukasik, J.O. (2006) Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. *Appl Environ Microbiol* **72**, 7567-7574 Briefly, water will be filtered and filers will be incubated on mEI Agar (as for EPA Method 1600). Incubation time will be extended to increase growth, and colonies of *Enterococcus* will then be washed from the filters. DNA will be extracted from the sample and PCR will be performed. Primers specific for the *esp* gene will be used and the products will be separated on a gel. The expected PCR product is 680 bp. A no-DNA PCR negative control, an extraction blank and a positive control of *Enterococcus faecium* C68 DNA will be included in with each batch.

A listing of analytical methods and equipment is provided in Table B.4.1. In the event of a failure in the analytical system, a TAMU-CC PL will be notified. The TAMU-CC Laboratory Supervisor, QAO, and PLs will then determine if the existing sample integrity is intact, if re-sampling can and should be done, or if data should be omitted.

Table B.4.1. Laboratory and Field Analytical Methods and Equipment.

Parameter	Method[1]	Equipment Used
Laboratory Parameters		
Enterococcus enumeration	EPA 1600	Incubator, filtration apparatus
Carbon source utilization profiles	Biolog instructions	Microbial Identification System and accessories, incubator etc.
Antibiotic resistance profiles	CLSI	BIOMIC image analysis system, disk dispensers, incubator
Esp gene detection	USF/TAMU-CC SOP from McQuiag, 2006	PCR – thermocycler, microcentrifuge, heating block, hood, gel apparatus, UV light
Field Parameters		
Dissolved Oxygen	EPA 360.1	YSI Multiprobe
Potential Hydrogen	EPA 150.1	YSI Multiprobe
Specific Conductance	EPA 120.1	YSI Multiprobe
Water Temperature	EPA 170.1	YSI Multiprobe
Flow	TCEQ SWQM	Global Water FlowProbe, Pygmy Flow Meter, Price Flow Meter, SonTek FlowTracker, or RDI- Acoustic Doppler Current Profiler

[1] Some methods are modified by TAMU-CC as outlined in Table A.7.1(See SOPs in Appendix D).
EPA = Methods for Chemical Analysis of Water and Wastes, March 1983
SM = Standard Methods for Examination of Water and Wastewater, 21st edition
TCEQ SWQM = TCEQ Surface Water Quality Monitoring Procedures, Volume 1 (RG-415)

Standards Traceability

All standards used in the laboratory are traceable to certified reference materials. Standards preparation is fully documented and maintained in a standards log book.

The use of standards and reagents are documented when used in preparation and analytical logs. Each documentation includes traceability to purchased stocks, reference to the method of preparation, including concentration, amount used and lot number, date prepared, expiration date and preparer's initials or signature. The reagent bottle is labeled with concentration, date of preparation, expiration date, storage requirements, safety considerations, and a unique identifier that traces the reagent to the standards and reagents log book entry.

Analytical Method Modification

Only data generated using approved analytical methodologies as specified in this QAPP will be used as direct data for this project. Requests for method modifications will be documented and submitted for approval to the TSSWCB. Work using modified methods will begin only after the modified procedures have been approved.

Deficiencies, Nonconformances and Corrective Action Related to Analytical Methods

Deficiencies are defined as unauthorized deviations from procedures documented in the QAPP. Nonconformances are deficiencies that affect quality and render data unacceptable or indeterminate. Deficiencies related to field and laboratory measurement systems include but are not limited to instrument malfunctions, blank contamination, quality control sample failures, etc.

Deficiencies are documented in logbooks and field data sheets by field or laboratory staff and reported to the field/laboratory supervisor. The supervisor will notify the TAMU-CC QAO. If the situation requires an immediate decision concerning data quality or quantity, a TAMU-CC PL will be notified within 24 hours. The TAMU-CC PL will notify the TAMU-CC QAO of the potential nonconformance. The TAMU-CC QAO will record and track the CAR to document the deficiency.

The TAMU-CC QAO, in consultation as appropriate with the TAMU-CC PLs, will determine if the deficiency constitutes a nonconformance based on best professional judgment. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TAMU-CC PLs in consultation with TAMU-CC QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TAMU-CC QAO.

CARs document: root cause(s); programmatic impact(s); specific corrective action(s) to address the deficiency; action(s) prevent recurrence; individual(s) responsible for each action; the timetable for completion of each action; and, the means by which completion of each corrective action will be documented. The TSSWCB will be notified of excursions that affect data quality with QPRs. All CARS will be submitted with QPRs. In addition, significant conditions (i.e.,

situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported to TSSWCB immediately.

Section B.5 Quality Control Requirements

Sampling Quality Control Requirements and Acceptability Criteria

Table A.7.1 in Section A7 lists the required accuracy, precision, and completeness limits for the parameters of interest. It is the responsibility of the Project Leaders to verify that the data are representative. The Project Leaders also have the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents requiring corrective action will be documented through use of CARs (Appendix A). Laboratory systems audits and monitoring systems audits will be conducted by the TSSWCB QAO or their designee as detailed in Table C.1.1.

The minimum Field QC Requirements are outlined in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). Specific requirements are outlined below. Field QC Samples are reported with the data report (See Section A9 and C2).

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

Method Specific QC requirements – QC samples, other than those specified later this section, are run (e.g., sample duplicates, positive control, negative control, and media blank) as specified in the methods. The requirements for these samples, their acceptance criteria or instructions for establishing criteria, and corrective actions are method-specific.

Detailed laboratory QC requirements are contained within each individual method and laboratory quality assurance manuals (QAMs). The minimum requirements that all participants abide by are stated below. Lab QC sample results are reported with the laboratory data report (see Section C.2 and Section A.9).

Limit of Quantitation (LOQ) – The laboratory will analyze a calibration standard (if applicable) at the LOQ on each day that samples are analyzed. Calibrations including the standard at the LOQ will meet the calibration requirements of the analytical method or corrective action will be implemented. LOQ is not applicable for bacteria.

Lab QC samples are prepared and analyzed in batches, which are defined as follows:

Batches are environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch is composed of one to 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or concentrates) that are analyzed together as a group. An analytical batch can include prepared samples originating from various environmental matrices and can exceed 20 samples.

Laboratory duplicate - Laboratory duplicates are used to assess precision. A bacteriological duplicate is considered to be a special type of laboratory duplicate. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Precision is calculated based on precision criterion. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair.

The method to be used for calculating precision is the one outlined in Standard Methods for the Examination of Water and Wastewater, 21st Edition, Section 9020-B, "QA/QC - Intralaboratory QC Guidelines." This criterion applies to bacteriological duplicates with concentrations >10 org/100 mL.

$$RPDbacteria = (\log X_1 - \log X_2)$$

The RPDbacteria should be lower than 3.27 ΣRlog/n, where Rlog is the difference in the natural log of the duplicates for the first 15 positivie samples. The specifications for bacteriological duplicates in Table A.7.1 apply to samples with concentrations > 10 cfu/100mL.

Precision for BST techniques will follow the specific method QC (see Additional method specific QC requirements below).

Matrix spikes (MS) - (not applicable)

AWRL /Reporting Limit Verification - The laboratory's reporting limit will be at or below the AWRL. (Verification not applicable)

Laboratory equipment blank – (not applicable)

Method Blank - A method blank is an analyte-free matrix to which all reagents are added in the same volumes or proportions as used in the sample processing and analyzed with each preparatory and analytical batch. The method blank is carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the laboratory's reporting limit. For very high level analyses, blank value should be less then 5% of the lowest value of the preparatory (if applicable) and analytical batch or corrective action will be implemented.

The equivalent quality control for bacteriological membrane filtration methods follows *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008). For each membrane filter test sterility of the media, petri dishes, membrane filters, dilution water and apparatus will be checked using about 20 ml sterile water. The analysis of equipment blanks should yield values less than the minimum analytical limit (1 colony per volume). A blank is run at the start and end of each sample analyzed. Normally data from samples with growth on blanks will be omitted; however, in cases where extremely high levels of bacteria are present in the sample, the blank run at the

end of the group should have less than 1% of the colonies on the sample filter of the highest volume filtered. Corrective action will be implemented if these values are exceeded.

Additional method specific QC requirements

Additional QC samples will be run (e.g., positive controls, negative controls for each selective medium lot and positive controls and sterility checks for all batches of media) as specified in Section 9020 B. *Standard Methods for the Examination of Water and Wastewater* (21st Edition, 2005) American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) 2005 and the 2003 NELAC Standard. Recommended positive and negative control cultures for enterococci will be used as per *Standard Methods* Table 9020:V. The controls selected will be *Streptococcus faecalis* ATCC 29212 and *Streptococcus salvarius* ATCC 13419. The requirements for these samples, their acceptance criteria, and corrective action are method-specific. A media log sheet showing date, medium, volume, and initials will be kept for all media prepared. All inoculated plates, tubes, broths etc. will be autoclaved in biohazard bags with indicator tape, for at least 30 minutes (121 °C) prior to disposal. Media which either supports growth of a negative control, does not support growth of the positive control or fails the sterility check will be discarded.

Quality control for CSU will follow the protocol described in the MicroLog™ System Release 4.0 User Guide (Biolog 1999a). Each GP2 MicroPlate™ has been tested and must have met internal quality control standards before being released for sale. A set of three gram-positive control strains: *Corynebacterium minutissimum* (ATCC 23348), *Rhodococcus equi* (ATCC 6939), and *Staphylococcus aureus* (ATCC 12600), will be streaked onto BUG plates, inoculated onto GP2 MicroPlates™, and analyzed with the Biolog MicroStation™ Reader for quality control purposes. Positive (*Enterococcus faecalis*) and negative (*Enterobacter aerogenes*) controls will be performed on each new batch of mEnterococcus medium.

Quality control for ARP will follow CLSI Performance Standards (2006, 2008). For ARP 10% of the isolates are run in duplicate. The objective for precision for ARP in this study is for the zones of inhibition for each of the duplicates for ARP to be identical, thus indicating the same animal source. An acceptable range in zone diameters for duplicates will be considered +/-3 mm. If this range is exceeded for more than 1 antibiotic the TAMU-CC QAO will examine the data for that isolate, and professional judgment will be used to determine whether that sample will be omitted from the database or if any other course of action is warranted e.g. re-training of analyst.

Quality control for *esp* analysis will follow the USF/TAMU-CC SOP and the McQuiag et al paper (2006). A positive control (*Enterococcus faecium* strain which contains the *esp* gene), and negative controls (a no-DNA sample, an extraction blank containing only buffer) will be included in each analytical batch.

Deficiencies, Nonconformances and Corrective Action Related to Quality Control

Deficiencies are defined as unauthorized deviation from procedures documented in the QAPP. Nonconformances are deficiencies that affect quality and render the data unacceptable or indeterminate. Deficiencies related to Quality Control include but are not limited to quality control sample failures.

Deficiencies are documented in logbooks, field data sheets or laboratory data sheets by field or laboratory staff and are reported by the Laboratory/Field Supervisor. The supervisor will notify the TAMU-CC QAO. If the situation requires an immediate decision concerning data quality or quantity, a TAMU-CC PL will be notified within 24 hours. The TAMU-CC PL will notify the TAMU-CC QAO of the potential nonconformance. The TAMU-CC QAO will record and track the CAR to document the deficiency.

The TAMU-CC QAO, in consultation as appropriate with the TAMU-CC PLs will determine if the deficiency constitutes a nonconformance based on best professional judgement. If it is determined the activity or item in question does not affect data quality and therefore is not a valid nonconformance, the CAR will be completed accordingly and closed. If it is determined that a nonconformance does exist, the TAMU-CC PLs in consultation with TAMU-CC QAO will determine the disposition of the nonconforming activity or item and necessary corrective action(s); results will be documented by completion of a CAR, which is retained by the TAMU-CC QAO.

CARs document: root cause(s); programmatic impact(s); specific corrective action(s) to address the deficiency; action(s) prevent recurrence; individual(s) responsible for each action; the timetable for completion of each action; and, the means by which completion of each corrective action will be documented. The TSSWCB will be notified of excursions that affect data quality with QPRs. All CARs will be submitted with QPRs. In addition, significant conditions (i.e., situations that, if uncorrected, could have a serious effect on safety or validity or integrity of data) will be reported to the TSSWCB immediately.

Section B.6 Equipment Testing, Inspection, & Maintenance Requirements

All sampling equipment testing and maintenance requirements are detailed in the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (October 2008).

Instrument/Equipment is inspected and tested upon receipt and is assured appropriate for use. Equipment records are kept on all field equipment and a supply of critical spare parts is maintained by the TAMU-CC Field Supervisor, or designee.Initial acceptance occurs at TAMU-CC Central Receiving by a designated employee who receives and signs for the materials. Acceptance criteria are detailed in the TAMU-CC Purchasing Department Policy and Procedure Handbook Revised Jan 31, 2005. Packages and their contents are reviewed to ensure that the shipment is complete. Items are then delivered to the appropriate analyst or manager. A second inspection is conducted by the QA Officer, Laboratory Supervisor or Project Leaders during which the equipment is tested following manufacturer's instructions to ensure equipment meets specifications.

All laboratory tools, gauges, instrument, and equipment testing and maintenance requirements are contained within laboratory QAM(s) and are inspected and maintained according to manufacturer specifications and based on Standard Methods Section 9020 B.3 and 9030 B. by appropriate laboratory personnel under the supervision of the laboratory supervisor Testing and maintenance records are maintained and are available for inspection by the TSSWCB. Instruments may include, but are not limited to, water baths, ovens, autoclaves, incubators, refrigerators, double distillation water unit, freezers, balances, pH meter, membrane filtration equipment, thermometers, media dispensing apparatus, centrifuges, safety cabinet, water bath, microscopes, UV lamp, spectrophotometer, computers, BIO-MIC automated plate reader system, Microbial Identification System, pipettes, bunsen burners, dilution bottles, and sample bottles. Critical spare parts for essential equipment are maintained to prevent downtime. Any deficiencies will be noted and how these deficiencies were resolved as part of routine maintenance records. If during routine maintenance of laboratory equipment, it is found that sample integrity may be in question, a CAR will be filled out for the samples impacted.

Section B.7 Instrument Calibration and Frequency

Field equipment calibration requirements are contained in the TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue (October 2008). Post calibration error limits and the disposition resulting from error are adhered to. Data not meeting post-error limit requirements invalidates associated data collected subsequent to the pre-calibration and will not be used for evaluation of project objectives.

Detailed laboratory calibrations are contained within the QAM(s). The laboratory QAM identifies all tools, gauges, instruments, and other sampling, measuring, and test equipment used for data collection activities affecting quality that must be controlled and, at specified periods, calibrated to maintain bias within specified limits. Calibration records are maintained and are available for inspection by the TSSWCB. Equipment requiring periodic calibrations include, but are not limited to, thermometers, pH meters, balances, incubators, turbidity meters, BIO-MIC system and analytical instruments. Autoclave performance is verified monthly following *Standard Methods* 9020 B. Intra-laboratory quality control guidelines (APHA, 2005). Biological safety cabinets and chemical hoods are certified annually.

Section B.8 Inspection/Acceptance Requirements for Supplies and Consumables

All new batches of field and laboratory supplies are inspected and tested before use to ensure that they are adequate and not contaminated. All standards, reagents, media, plates, filters and other consumables are purchased from manufacturers with performance guarantees, and are inspected upon receipt for damage, missing parts, expiration date and storage and handling requirements. Labels on reagents, chemicals and standards are examined to ensure they are of appropriate quality, initialed by a staff member and marked with receipt date. All supplies will be stored as per manufacturer labeling and discarded past expiration date. The laboratory QAM provides additional details on acceptance requirements for laboratory supplies and consumables.

Section B.9 Data Acquisition Requirements (Non-direct Measurements)

All required data to be used for this project will be collected in accordance with this QAPP. All data used in the actual assessment will be new data based on the samples taken and analyzed during the study, with the exception of a portion of the profiles in the BST known source library (see below).

Additional data which will be used during this study are as follows:

- Precipitation data from the NOAA National Weather Service to determine amounts and dates of rainfall in the watershed needed for scheduling sampling following rainfall events.
- Flow data from USGS gage station (Gage #08211520) on Merritt Road to provide flow information for the creek, during the study, in addition to measurements made during field collections events under this QAPP.
- Land use and sanitary survey information to determine potential source animals and appropriate locations for fecal sample collection, collated by the PLs under
  - Oso Creek and Oso Bay Bacteria TMDL Project. Quality Assurance Project Plan. TCEQ project USEPA QTRAK #05-246 (amended for and extended for year 2) 2005-2006

  and from
  - Comments from multiple Oso Creek/Oso Bay TMDL Stakeholder meetings including one held January 28, 2008 with the TSSWCB (detailed in April 2008 QPR)
- CSU profiles for Enterococcus to supplement the library to be developed under this QAPP, from a special study plan approved and funded by the TGLO Investigation to evaluate use of Biolog microplates (carbon source utilization) as a bacterial source tracking technique for Texas coastal waters. 2002-2003.

  Data obtained from CSU profiles of unknown source enterococci in accordance with this QAPP will be compared with a library that will include some existing data (~ 400 CSU profiles for Enterococcus) in a database stored at TAMU-CC which was previously collected, reviewed and accepted in accordance with a previously approved work plan (Texas General Land Office 2002 – see above). Data from these isolates from animal samples collected within the Coastal Bend area will be judged to be acceptable. Isolates have been stored at -80°C and will be re-grown for ARP analyses. A limitation of the data is that the temporal stability of Enterococcus characteristics is unknown; however, funding available precludes construction of a completely new library. Data from the existing isolates will be compared with the new isolates and if they are not consistent a decision will be made whether to use only isolates collected during the study described in this QAPP. The library size would however, then be limited. The existing library of isolates will be significantly expanded with isolates obtained under this QAPP.

Additional data may be incorporated into the study to provide background information which will assist in achieving the goals of the project as follows:

- Bacteria concentrations measured in previous and on-going agricultural NPS studies:
  - TSSWCB Project 02-13: Estimation of Water-Quality Constituent Loadings from Agricultural Croplands in the Oso Creek Watershed. Quality Assurance Project Plan.

Clean Water Act Section 319(h) Nonpoint Source Pollution Control Program Project Texas State Soil and Water Conservation Board Project No. 02-13 Texas Agricultural Experiment Station. Effective Period: July 2005 to December 2006

and

- TSSWCB Project 07-07: Assessment of Non Point Source Pollution from Cropland in the Oso Bay Watershed. Quality Assurance Project Plan. Clean Water Act Section 319(h) Nonpoint Source Pollution Control Program Project in cooperation with Texas State Soil and Water Conservation Board and U.S. Environmental Protection Agency Effective Period: September 2007 to August 2009.

- Bacteria concentrations used to establish current bacterial loadings in the Oso Creek watershed measured under
  - Nueces River Authority Quality Assurance Project Plans. Clean Rivers Program Monitoring Operations Division. Texas Commission on Environmental Quality. Effective Periods: FY 2008 to FY 2009, FY 2007-2008, and annually for previous years
  - Oso Creek and Oso Bay Bacteria TMDL Project. Quality Assurance Project Plan. TCEQ project USEPA QTRAK #05-246 (amended for and extended for year 2) 2005-2006

- Bacteria concentrations and location of outfalls in the upper Oso Creek, currently being collected under
  - CBBEP Oso Creek Bacteria Contamination Investigation. Quality Assurance Project Plan. CBBEP Contract Number: 0816. 2008-2009.

- Nutrient levels and groundwater levels in the watershed. Temperature is being used as a proxy for effective flow to establish surface and groundwater connections. Data being collected under
  - CBBEP Oso Watershed Characterization – Ground Water Monitoring Quality Assurance Project Plan. CBBEP Project No. 0541. 2008-2009

- Bacteria source tracking information on use of ARA vs. other methods from data collected under
  - TSSWCB Project 02-10: Development of an *E. coli* bacterial source tracking library and assessment of bacterial soruces impacting Lake Waco and Lake Belton. Quality Assurance Project Plan. Clean Water Act Section 319(h) Nonpoint Source Pollution Control Program Project Texas State Soil and Water Conservation Board Project No. 02-13 Texas Agricultural Experiment Station. Effective Period: July 2003 to August 2004, extended.

Section B.10 Data Management

Control of all field and laboratory documents is addressed in the TAMU-CC EML SOP for Data Control Procedures. Electronic data is addressed in the SOP for Protection of Electronic Data. Data management procedures not covered in these SOPs are addressed in the TAMU-CC EML QAM. All of the aforementioned documents are available for review upon request.

Data for this project will be produced at TAMU-CC laboratories. Data will be reviewed following Table D.2.1 Data Review, Verification, and Validation Procedures.

Data collection begins with the collection of field samples. Field staff will measure dissolved oxygen, pH, water temperature, flow rate, salinity, and specific conductance at each stream site, using calibrated multi-sonde equipment.. Measurements read from the instruments will be recorded on the field data sheet. Samples will be collected at the site, and an identification number (either a sample identification number or a site code) will be written in permanent marker on the outside of the container (dependent on sample type). The containers are placed in an ice chest for transportation to the laboratory. Field personnel will record all samples and field observations on field data sheets by hand (Appendix B). Field data will be transferred by lab personnel to Excel spreadsheets. All transfer of data to electronic format will be 100% proof read by a second analyst. COC forms are used for each sampling event (Appendix C) to record water sample identification parameters and to document the submission of samples from the field staff to the analytical laboratory staff. Each COC has space to record data for numerous separate samples. All entries onto the COC forms will either be typed or completed in ink, with any changes made by crossing out the original entry, which should still be legible, and initialing and dating the new entry. Field data sheets and COCs are copied and stored as hard copy at TAMU-CC for at least five years.

All field samples are logged into the lab upon receipt. COC forms will be checked by lab personnel for number of samples, I.D. number, signatures, dates and type of analysis specified. A unique sample identification number is given to each bacteriological sample upon receipt. This unique identifier will follow the sample throughout the analytical process. The QAO will be notified if any discrepancies are found and laboratory analysis will not occur until proper corrections are made. All samples will be stored at 4ºC until analysis.

Samples analyzed in the laboratory generate the next level of data. This bacteriological data is hand recorded by lab personnel on data sheets and proof read. Proof reading in both cases involves a 100% check of each handwritten number. Enumerated bacteriological data will be manually entered into the database system for electronic storage. The electronic database will be created in Microsoft® Excel and/or Access software on an IBM-compatible microcomputer with the Windows XP Operating System The project database will be maintained on the computer's hard drive, which is also simultaneously saved in an external network folder. Carbon source utilization data is produced electronically and as printouts from the BIOLOG software previously

installed at TAMU-CC. Antibiotic resistance analysis data is produced electronically and as printouts from the BIOMIC software previously installed at TAMU-CC. These two instruments have separate hard drives, used exclusively for this purpose. The esp analysis will generate gels with or without a 680 bp product. Each gel result will be scored as a positive or negative result on data sheets and this information will be entered into the database system for electronic storage. All data sheets will be copied and stored as hard copy in two locations at TAMU-CC for at least five years. Data will be transferred by lab personnel to spreadsheets for statistical analysis using SPSS. An electronic back-up of spreadsheets will be made at least monthly on CD-ROM. All transfer of data from one format to another will be proof read by a second analyst.

All project computer files will be backed up using CD-ROMs and an external hard drive at least monthly. These files will be stored for at least five years at TAMU-CC. Current data files will be backed up on external hard drives weekly. At least 10% of all data manually entered in the database will be reviewed for accuracy by the QAO to ensure that there are no transcription errors. Hard copies of data will be printed and housed at TAMU-CC for a period of five years.

At the conclusion of the project a final report will be sent to TSSWCB which will include the results of the field sampling and analyses for enterococci and field parameters, and BST results in tabular form and Excel or SPSS spreadsheets on a CD-ROM. Statistical analyses will be summarized in tables.

Section C

Section C.1 Assessments and Response Actions

The following table presents the types of assessments and response actions for data collection activities applicable to this project (Table C.1.1).

Table C.1.1. Assessments and Response Actions.

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	TAMU-CC Project Leaders	Monitoring of the project status and records to ensure requirements are being fulfilled	Report to TSSWCB in QPRs
Monitoring Systems Audit of TAMU-CC	minimum of one per life of project	TSSWCB QAO	The assessment will be tailored in accordance with objectives needed to assure compliance with the QAPP. Field sampling, handling and measurement; facility review; and data management as they relate to the project	30 days to respond in writing to the TSSWCB to address corrective actions
Laboratory Inspection	minimum of one per life of project	TSSWCB QAO	Analytical and quality control procedures employed at the TAMU-CC laboratory	30 days to respond in writing to TSSWCB to address corrective actions
Laboratory Management Review	Annually	TAMU-CC QAO	Conduct management reviews of the laboratory's quality system to ensure its effectiveness	Not applicable
Laboratory Internal Audits	Annually	TAMU-CC Laboratory QAO	Conduct internal audits of the quality system to verify that activities comply with the quality system Standard	30 days to respond in writing to QAO to address corrective actions

Corrective Action

The TAMU-CC PLs are responsible for implementing and tracking corrective action procedures resulting from audit findings outlined in any internal or external audit report. The TAMU-CC QAO will maintain records of audit findings and corrective actions. Internal audit reports will be made available to the TSSWCB upon request. External audits conducted by the TSSWCB will include CARs of any findings directly to the TSSWCB.

Section C.2 Reports to Management

Laboratory Data Reports

Laboratory data reports contain the results of all specified QC measures listed in Section B.5, including but not limited to laboratory duplicates, field splits, and method blanks, as applicable for bacteriological samples. This information is reviewed by the TAMU-CC QAO and compared to the pre-specified acceptance criteria to determine acceptability of data before forwarding to the TAMU-CC Project Leaders. This information is available for inspection by the TSSWCB.

Reports to TAMU-CC Project Management

The TAMU-CC QAO will provide laboratory and QA data reports and an update on project status to the PLs after each sampling event. Any QA issues will be referred immediately to the Lab QA Officer who will review the issue and all documentation, and notify the TAMU-CC PLs either verbally or by email. In cases of significant QA issues, project delays etc. the PLs will meet with the QAO to determine appropriate action, following QAPP requirements. All data sheets will be reviewed after each sampling event by the PLs to determine acceptability of data.

Reports to TSSWCB Project Management

Quarterly Progress Report – Summarizes TAMU-CC's activities for each task; reports problems, delays, and corrective actions; and outlines the status of each task's deliverables. Report written by the TAMU-CC PLs.

Monitoring System Audit Response – TAMU-CC will respond in writing to the TSSWCB within 30 days upon receipt of a monitoring system audit report to address corrective actions. Response written by the TAMU-CC QAO.

Laboratory System Audit Response – TAMU-CC will respond in writing to the TSSWCB within 30 days upon receipt of a laboratory system audit report to address corrective actions. Response written by the TAMU-CC QAO.

Final Project Report – Summarizes TAMU-CC's activities for the entire project period including a description and documentation of major project activities; evaluation of project results and environmental benefits; and a conclusion. Report written by or under the guidance of the TAMU-CC PLs with assistance from other staff members. The intent is for enterococci data to be submitted by TSSWCB to TCEQ.

Section D

Section D.1 Data Review, Validation, and Verification

For the purposes of this document, data verification is a systematic process for evaluating performance and compliance of a set of data to ascertain its completeness, correctness, and consistency using the methods and criteria defined in the QAPP. Validation means those processes taken independently of the data-generation processes to evaluate the technical usability of the verified data with respect to the planned objectives or intention of the project. Additionally, validation can provide a level of overall confidence in the reporting of the data based on the methods used.

All data obtained from field and laboratory measurements will be reviewed and verified for conformance to project requirements, and then validated against the DQOs listed in Section A7. Only those data that are supported by appropriate quality control data and meet the measurement performance specification defined for this project will be considered acceptable and used in the project.

The procedures for verification and validation of data are described in Section D.1. The TAMU-CC Field/Laboratory Supervisor is responsible for ensuring that field data are properly reviewed and verified for integrity and that laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. The TAMU-CC QAO and Project Leaders will be responsible for ensuring that all data are properly reviewed and verified, and submitted in the required format to the project database. The TAMU-CC QAO is responsible for validating a minimum of 10% of the data produced in each task. Finally, the TAMU-CC Project Leaders, with the concurrence of the TAMU-CC QAO, are responsible for validating that all data collected and analyzed meet the objectives of the project.

All field and laboratory data will be reviewed and verified for integrity and continuity, reasonableness, and conformance to project requirements, and then validated against the project objectives and measurement performance specifications which are listed in Section A7. Data that are supported by appropriate quality control data and meet the measurement performance specifications defined for this project will be considered acceptable will be used in evaluating project objectives for the final report.

Section D.2 Validation and Verification Methods

All data will be verified to ensure they are representative of the samples analyzed and locations where measurements were made, and that the data and associated quality control data conform to project specifications. The staff and management of the respective field, laboratory, and data management tasks are responsible for the integrity, validation and verification of the data each task generates or handles throughout each process (Table D.2.1). The field and laboratory QA tasks ensure the verification of raw data, electronically generated data, and data on chain-of-custody forms and hard copy output from instruments.

Verification, validation, and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the manager of the task. The data to be verified are evaluated against project performance specifications (Table A.7.1 and Table D.2.1) and are checked for errors, especially errors in transcription, calculations, and data input. If a question arises or an error is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues that can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork.

If an issue cannot be corrected, the task manager consults with higher level project management to establish the appropriate course of action, or the data associated with the issue are rejected.

The TAMU-CC Project Leaders and QAO are each responsible for validating that the verified data are scientifically valid, defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to TSSWCB. One element of the validation process involves evaluating the data again for anomalies. The manager of the task associated with the suspected data errors or anomalous data must address these issues before data validation can be completed.

A second element of the validation process is consideration of any findings identified during a laboratory or monitoring systems audit conducted by the TSSWCB QAO. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, the TAMU-CC Project Leaders, with the concurrence of the TAMU-CC QAO, validates that the data meet the DQOs of the project and are suitable for meeting project objectives for the TSSWCB.

Table D.2.1. Data Review, Verification, and Validation Procedures.

Field Data Review	**Responsibility**
Field data reviewed for conformance with data collection, sample handling and chain of custody, analytical and QC requirements	TAMU-CC Field Supervisor
Post-calibrations checked to ensure compliance with error limits	TAMU-CC Field Supervisor
Field data calculated, reduced, and transcribed correctly	TAMU-CC Field Supervisor
Laboratory Data Review	
Laboratory data reviewed for conformance with data collection, sample handling and chain of custody, analytical and QC requirements to include documentation, holding times, sample receipt, sample preparation, sample analysis, project and program QC results, and reporting	TAMU-CC Laboratory Supervisor
Laboratory data calculated, reduced, and transcribed correctly	TAMU-CC Laboratory Supervisor
Reporting limits consistent with requirements for Ambient Water Reporting Limits.	TAMU-CC Laboratory Supervisor
Analytical data documentation evaluated for consistency, reasonableness and/or improper practices	TAMU-CC Laboratory Supervisor
Analytical QC information evaluated to determine impact on individual analyses	TAMU-CC Laboratory Supervisor
All laboratory samples analyzed for all parameters	TAMU-CC Laboratory Supervisor
Data Set Review	
Data reported has all required information as described in Section A9 of the QAPP	TAMU-CC QAO
Confirmation that field and lab data have been reviewed	TAMU-CC QAO
Data set (to include field and laboratory data) evaluated for reasonableness and if corollary data agree	TAMU-CC PLs
Outliers confirmed and documented	TAMU-CC QAO and PLs
Field QC acceptable (e.g., field splits)	TAMU-CC QAO
Sampling and analytical data gaps checked and documented	TAMU-CC QAO and PLs
Verification and validation confirmed. Data meets conditions of end use and are reportable	TAMU-CC PLs

Section D.3 Reconciliation with Data Quality Objectives

Data produced in this project, and data collected under other TAMU-CC projects or by other organizations (e.g., TCEQ, USGS, CBBEP), will be analyzed and reconciled with project data quality requirements. Data meeting project requirements will be provided to meet informational needs on nonpoint sources of enterococci in the upstream section of Oso Creek to state agencies and local planning entities in support of development of the TMDL and I-Plan for Oso Creek. Only data meeting all QA requirements will be submitted to the TSSWCB.

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Warning: When references are made to documents that are not attached to the QAPP, the Project Manager/QAO of the TAMU-CC will assume responsibility for compliance of the documentation with the procedures and requirements set forth in the QAPP.

Appendix A. Corrective Action Report

Corrective Action Report
TSSWCB Project 07-13

CAR #:_____

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

State the nature of the problem, nonconformance, or out-of-control situation:
_____
_____
_____
_____
_____

Possible causes:
_____
_____
_____

Recommended corrective action:
_____
_____
_____

CAR routed to:_____
Received by:_____

Corrective Actions taken:
_____
_____
_____
_____

Has problem been corrected?: YES NO

Immediate Supervisor:_____

Project Leader:_____

Quality Assurance Officer:_____

Appendix B. Field Data Sheet

Texas A&M University-Corpus Christi
The Island University

Environmental Microbiology Laboratory
Field Data Sheet

Date: _____ YSI/Hydrolab Multiprobe #: _____

Sampling Location: _____ Station ID: _____

Time Collected: _____ Time In: _____ Time Out: _____

Lat/ Long :_____ Truck Out: _____ Truck In: _____

Sample Collector Initials: _____ Monitor(s) Name (s): _____

STORET CODE	VALUE	Parameter	Comments
	NA	**Depth Sample Collected (cm)**	
00020		**Air Temp (°C)**	
89965		**Wind Intensity** 1=Calm (0), 2=Slight (1 to 7), 3=Moderate (8 to 18), 4=Strong (19+)	
89010		**Wind Direction** 1=N, 2=S, 3=E, 4=W, 5=NE, 6=SE, 7=NW, 8=SW	
89966		**Present Weather** 1=Clear (0 to 25%), 2=Cloudy (25 to 99%), 3=Overcast (100%), 4=Rain	
00010		**Water Temp (°C)**	
00094	NA	**Conductivity (µmhos/cm)**	
00480		**Salinity (ppt)**	
00300	NA	**DO (mg/L)**	
00400	NA	**pH (s.u.)**	
00078	NA	**Secchi Disk (meters)**	
89969		**Water Color** 1=Brown, 2=Reddish, 3=Green, 4=Black, 5=Clear, 6=Other	
89971		**Water Odor** 1=Sewage, 2=Oily/Chemical, 3=Rotten Eggs, 4=Musky, 5=Fishy, 6=None, 7=Other	
89968		**Water Surface** 1=Calm, 2=Ripples, 3=Waves, 4=White Caps	
89972		**Tide Stage** 1=Low, 2=Falling, 3=Slack, 4=Rising, 5=High	
72053		**Days Since Last Rainfall**	
82553		**Rainfall (Inches past 1 day)**	
82554		**Rainfall (Inches past 7days)**	

H Human Use (Fisherman/Swimmers/Kayakers/Windsurfers):

_____

_____

Other Comments:

_____

_____

Appendix C. Chain-of-Custody Form

Texas A&M University-Corpus Christi
The Island University

Chain of Custody Form

Department of Life Sciences
6300 Ocean Drive
Corpus Christi, Texas 78412

P.I.: Dr. Joanna Mott
Texas A&M University–Corpus Christi, Unit 5802
Environmental Microbiology Lab., CS 237

Lab Analysis Request

Project Leader:	Phone:	Fax:	Project Name/Location:	Project No.
	361-825-3262	361-825-3719		

Samplers (sign):		Total Containers	Media Code	Matrix		Preserved (w/ice)		Sampling		Gel Pulse Field Electrophoresis	Fecal Coliforms SM20 9222-D	Escherichia coli SM 20 9213 D	Enterococci EPA 1600	Clostridium perfringens	mHPC SM20 9215-A	Epifluorescence SM20 9216-B	IDEXX® Enterolert™		TAMU-CC
Sampling Identification No:	Lab Only			Liquid	Soil	$Na_2S_2O_3$ Added Mark "X" on lid	Ice/Ref (Only)	Date	Time										**Remarks**

Relinquished by: (Print)	Date:	Time:	Received by: (Print)	Date:	Time:
(Sign)			(Sign)		

Appendix D. Bacteria Source Tracking SOP

Enterococcus DNA Extraction for *esp* Assay using the QIAamp Stool Mini Kit InhibitEX tablets

Courtesy Dr. V. Harwood Laboratory, University of South Florida

(updated by Dr. K. Gordon 12/08/08)

I. Sample Processing (McQuaig *et al.* 2006)

- Filter 300ml of each sample through a 0.45 µm nitrocellulose filter.
- Incubate filters on mEI agar for 48h at 41°C.

II. Controls

- Prepare a method blank (MB) consisting of 300ml sterile water filtered through a 0.45 µm nitrocellulose filter.
- Prepare two positive controls, each of which will be spiked with 100µl of a 10^{-5} dilution of an overnight culture of *Enterococcus faecium* C68, which contains the esp gene. These controls are termed Spike 1 (SP1) and spike 2 (SP2). SP1 is a 300 ml composite sample of each of the sites sampled while SP2 is 300 ml of buffered dilution water.
- Incubate filters on mEI agar for 48h at 41°C.

IMPORTANT NOTE: CHANGE GLOVES FOLLOWING <u>EACH</u> STEP!!!
USE BARRIER PIPET TIPS THROUGHOUT!

III. Enrichment Step (McQuaig *et al.* 2006)

Have ready 15 mL screw-cap tubes (1 per sample + positive control), each containing 5 ml azide dextrose broth (Difco). Lift filters containing enterococci colonies from mEI plates with sterile tweezers, crumble, and place into the top of the tube. Push the filter down with a sterile swab. Vortex vigorously and incubate for 3 hours at 41^{o}C with vigorous shaking to wash bacteria from the filters and enrich the culture.

IV. Preparation for Extraction

- Spray bench with (1) 70% ethanol and wipe, and (2) DNA Away and wipe
- Pre-heat 2 heating blocks: 1 to 95°C and 1 to 70°C
- Set out and label 1 filter spin column per sample and 1 for extraction control.
- Label 3 sets of 2 ml microcentrifuge tubes for (a) initial centrifugation, ASL and addition of InhibitEX tablet (b) Proteinase K and then transfer of inhibitEX supernatant and (c) final DNA elution step.
- Aliquot reagents into 15 ml or 50 ml sterile, screwcap tubes or microcentrifuge tube (proteinase K): ASL, AL, ethanol, proteinase K, AW1, AW2, AE.

V. Extraction (Modified from Manufacturers instructions)

- From each sample, pipet 1.8 ml into a 2 ml microcentrifuge tube. Excess culture can be stored at 4ºC in case of problems with assay.
- Centrifuge culture tubes at high speed in microcentrifuge[c] 2-3 min. to pellet.
- Decant the supernatant and resuspend the pellet in 1.2 ml **ASL lysis buffer** (Qiagen, Inc.).
- Vortex for 1 min or until homogenized. Also set up the extraction blank (ASL buffer only). Transfer tubes to heating block and incubate at 95ºC for 5 min.
- Add 1 **InhibitEX tablet** (Qiagen, Inc.) to each sample and vortex immediately and continuously for 1 min until tablet is completely suspended. Incubate for 1 min at room temperature to allow inhibitors to absorb to the InhibitEX.
- Centrifuge sample at full speed for 3 min to pellet inhibitors bound to InhibitEX.
- Pipet 200 µl of supernatant (be sure not to get any of the pellet) into a new microcentrifuge tube and add 15 µl of **Proteinase K** (Qiagen, Inc.).
- Add 200 µl **Buffer AL** (Qiagen, Inc.) and vortex for 15 s. **Note**: Do not add proteinase K directly to Buffer AL.
- Transfer microcentrifuge tubes to heating block. Incubate at 70°C for 10 minutes.
- Add 200 µl ice cold **absolute ethanol** and vortex the samples immediately.
- Transfer the resulting suspension to filter spin columns, using a pipet, followed by centrifugation at 10,000 × g for 1 min. Remove tubes from centrifuge SLOWLY to avoid wetting the column (this caveat applies to next steps). Discard collection tube.
- Place columns into new collection tubes and wash each column with 500 µl **buffer AW1** (Qiagen, Inc.) by centrifugation at 10,000 × g for 1 min. Discard collection tube.
- Place columns into new collection tubes and wash each column with 500 µl **buffer AW2** (Qiagen, Inc.) by centrifugation at 14,000 × g for 3 min. **Note:** It is crucial that the flow through not wet the column during transfer.
- Place each column in its CORRESPONDINGLY LABELED MICROCENTRIFUGE TUBE. Elute purified DNA from the columns with 200 µl **buffer AE** (Qiagen, Inc.) by centrifugation at 10,000 × g for 1 min. Store the eluate at -20ºC until used as PCR template.

VI. PCR

- Turn on the thermocycler; program set to go to 95°C and hold.
- Turn on UV light in PCR hood 15 min. before beginning. The hood should contain a vortex, pipettors, tips, microcentrifuge tubes (0.2 and 1.5 ml), aliquots of autoclaved, nanopure water.
- Take primers (aliquot of working solution) out to thaw on ice (be sure to mark the tube each time you thaw).
- Label tubes. Include a positive control and a blank tube
- Calculate cheat sheet for master mix composition (see below)
- Turn UV light off
- Clean hood and gloves with DNA Away.
- In hood, make master mix (~30 reactions can fit in a 1.5 ml tube)
- Put Taq and primers back in freezer.

- Vortex master mix.
- Dispense 45 µl master mix per tube in 0.2 ml tubes; keep tubes on ice.
- Add template to tubes on bench (NOT under hood) that has been cleaned with DNA Away. Always run a no-DNA PCR negative control in addition to extraction blank in addition to a positive reaction with *Enterococcus faecium* C68 DNA as the template.

Recipe per reaction using GoTaq Green Mix (Promega; Taq, dNTPs and buffer included)

- 25 µl JumpStart Mix
- 15 µl H_2O
- 2.5 µl forward primer (working concentration 10 mM; diluted 1:10 from 100 mM stock); 5'-TAT GAA AGC AAC AGC ACA AGT T-3'(Scott *et al.* 2005)
- 2.5 µl reverse primer (working concentration 10 mM; diluted 1:10 from 100 mM stock); 5'-ACG TCG AAA GTT CGA TTT CC-3'- (Hammerum and Jensen 2002)
- 5 µl template (15 – 60 ng/µl)

PCR Cycle:

- Initial 94°C for 2 min.

30 cycles of:

- 94°C 1 min
- 58°C 1 min
- 72°C 1 min

1 cycle of

- Final 72°C for 5 min

Hold at 4°C

VII. Electrophoresis

- Have ready a 2.0% agarose gel (1ul 1% EtBR added) in 1X TAE.
- Load the Promega 100 bp ladder in the first lane.
- Run the gel at 90 V for ~ 45 min.
- The expected product is 680 bp.

References

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TAMU-CC Antibiotic Resistance Analysis Protocol

Follow procedures of Clinical and Laboratory Standards Institute (CLSI)

CLSI (2006) Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard-Ninth Edition. CLSI document M2-A9.

CLSI (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Third Edition. CLSI document M31-A3.

CLSI (2006) Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S16

Quality Assurance/Quality Control Protocol

EQUIPMENT AND SUPPLIES

For data to be reliable, standard QA/QC must be followed in the lab. All equipment (i.e. incubators, biohood, refrigerators) and supplies used must be maintained according to QA/QC standards.

Antibiotic discs must be kept in the freezer at -14°C or below until needed (M2-A9, p9). Discs may be stored in refrigerator at 8°C or below. However, drugs from B-lactam class (AMC, AM) should be stored in fridge no longer than a week. Other labile antibiotics (IPM) should also remain frozen. A small working supply placed into the disk dispensers can be kept in the refrigerator as long as they are stored in a tightly sealed desiccated container. An individual antibiotic tube of each antibiotic may be kept in the refrigerator in case the dispenser needs to be changed and this will allow for a quick warming time. These antibiotics may only stay in the refrigerator for up to one week. If you must open a new box of antibiotic cartridges, look for the box that has the nearest expiration date. When a box is finished, please remember to update the Drug Log on BIOMIC. Always make sure to enter media and antibiotics in BIOMIC as they arrive. Log into BIOMIC, click on “logs”, and update the system. If a box of discs expires, let the project manager know. Do not throw expired discs away; notify lab manager.

Disc dispensers are kept in the refrigerator and must be taken out and allowed to reach room temperature before opening. The extra antibiotics may also be removed from the refrigerator in case you need to change the dispenser while plating. A metal desiccator should always be in the dispenser case. This desiccator has blue beads inside, which turn pink when saturated with moisture. If you notice the beads are pink, heat the desiccator at 121^{o}C for 2-3 hours in the grey, dry oven in the main lab (DO NOT USE THE AUTOCLAVE.). When using the dispensers and an "X" on the antibiotic disk in observed, the antibiotics must be changed. The dispensers should be cleaned each time the cartridges are changed. The cleaning protocol and recipes for reagents involved in this process follow below. Stock solutions of Sterile DI water and 3% disinfectant must be maintained and may be kept on the shelf for up to three weeks.

<u>To prepare sterile DI water:</u>
Fill two 1 liter flasks with NANOpure water.
Autoclave on a 15 minute cycle for sterility.

<u>To prepare a 3% disinfectant solution:</u>
Pour 30mL of Lysol disinfectant found into a 1 liter flask
Fill the remaining (970mL)with NANOpure water to make 1 liter.

<u>To prepare an 85% isopropyl solution:</u>
Pour 850mL isopropyl alcohol into a 1L flask.
Add 150mL reagent water to flask.
Note The 85% isopropyl alcohol must also be used to clean the dispensers, but this must be kept in the flammable cabinet and not as a stock solution on the shelf.

<u>To clean the stampers</u>

1. Once antibiotic canisters display an "X", they must be replaced. This "X" represents the last antibiotic in the sleeve of 50 antibiotics. Move switch on tamper to "unlock" position. Pull out and throw out empty canisters into biohazard trash bin.
2. Set out four empty (no media in them) 150 X 15 mm petri dishes. Fill one about halfway with Lysol® disinfectant solution. Fill the second halfway with 85% isopropyl solution. Fill the last 2 halfway with sterile DDI water.
3. Place stamper directly over first plate and push lever down completely. Make sure white dispenser tabs touch liquid. Leave submerged for 30 seconds.
4. Repeat with last 3 plates. Allow dispenser to air dry. The dispenser may be dried on the underneath side where the discs come out with sterile swabs.
5. Refill dispenser with appropriate antibiotic canisters. Once refilled, make sure all of the antibiotic canisters are pushed down and slide the switch into "lock" position.

CONTROLS

Controls are run with each set of samples and anytime the lot number of media, plates, or antibiotic discs is changed. The *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition* (or edition is current to date), indicate the control strains to be used. Check with this publication to see which ones are current for the type of bacterial samples being run. For *Enterococcus sp.* analysis, the minimal QC recommendations from CLSI is *Staphylococcus aureus* (ATCC 25923) (M100-S16, p52). Controls should be maintained on TSA slants in the culture fridge. These slants may be used for up to three months.

MEDIA PREPARATION

Media should be kept in the proper cabinet to avoid moisture. Directions for making media are on the bottles and described below. Media should be put back in its proper place when finished so that it can be located by everyone. Make sure to log the media for antibiotic resistance in the current media log sheets folder. When a new bottle of media is opened it should be noted in the Media Log in BIOMIC. The bottle of media with the closest expiration date should be opened. The date the media is opened needs to be written on the bottle along with the initials of the person who opened it. All media for this project has straightforward directions on the bottle. NANOpure water must be used and the pH must be checked for each flask of media made. Make sure the pH and conductivity of the NANOpure water has been checked before use.

Mueller Hinton Agar

Mueller Hinton agar is used for the plates used for the antibiotic analysis. Each isolate will require approximately 140mL (for two plates), plus additional plates for controls.

After autoclaving, set one flask under the biohood and the other flasks onto hot plates on low heat and low stir to keep them from solidifying.

Use 150x15mm petri plates when pouring Mueller Hinton agar. Do not throw away the bags for these. They will be used for storage once the plates are solidified.

Set each plate flat, and pour media just to the line in 150mm plates under the biohood (~60-70mL per plate). Do not stack the plates after pouring. Set the lid ajar so that the condensation may escape as the media solidifies. Condensation in the plates may dilute the concentration of the bacteria when plating.

Once the plates are solidified, invert the plates into the plastic bags.
Tape the bags shut, label the tape with the date, media type and project name and store media in the refrigerator. These plates are good for up to two weeks.

Place one plate immediately into 35^{o}C incubator and check for sterility after 24 hours.

TSB Broth
TSB broth is used in the sample preparation. Nutrient broth is also acceptable to use.

Each isolate will require 5mL for each tube and 3-4mL for the initial sample preparation. Additional broth will be required to adjust the turbidity of the sample and for preparing controls. Before autoclaving, pipette broth (1 tube for each isolate and control, with extras for emergency) into 16x125mm disposable test tubes (5mL/tube) and cap the tubes. Pour the remaining broth into 125mL flasks (50-75mL/flask). Place foil over the flasks or put screw-cap on (depending on flask in use). Place autoclave tape over the flasks and a long strip over the test tube caps.

Set media into the autoclave on a 15-minute liquid cycle.
Label all media with date, initials, and type of media. Refrigerate in media refrigerator until used (4-8°C). Flasks with screw-caps are good for up to 3 months. Flasks with foil only and disposable tubes are good for up to 2 weeks. Discard media if color change is noted or contamination occurs.

TSA Plates
TSA plates are needed for the preparation of samples. Samples will be streaked prior to inoculation of broth to ensure fresh growth. To maximize usage of media, up to eight streaks can be made per plate if plate is divided into segments.

TSA plates can be stored in fridge up to two weeks.

SAMPLE PREPARATION (Day before procedure)

When preparing samples you must allow them ample time to grow. You can use samples from slants or from cryofreeze. To transfer:

1. UV sterilize the hood for 15 minutes. Turn UV light off and clean working surfaces with Sporocidin.
2. Invert TSA plate and split into 8 even sections using a Sharpie. Label each section with a sample number.
3. Collect supplies. Sterile loops (1uL for cryovials, 10uL can be used for slants) or needles may be used to transfer the cells to the agar plates.
4. If using cells from cryo, remove only a few samples from the freezer at a time to **avoid thawing**. Continually thawing and refreezing may break cells and decrease viability of the sample.
5. Remember to **always use aseptic technique.** Take the vial from the freezer, open the cap to the vial and collect a small amount of the sample. Streak the cells onto a section of the TSA plate. Use the same procedure if collecting from slants. Place plates back into appropriate refrigerator and vials back into cryofreeze as soon as possible. Controls are usually taken from working cultures on TSA slants. Remove only the slants needed for transfer from the

refrigerator. Allow the cultures to come to room temperature before transferring to the TSA plate.

6. Set the samples into the rotator in Incubator 10 or in a rack in tabletop shaking incubator (35°C). Place them in the rotator in numerical order going clockwise from the "Start" sticker. **Log info** on the incubator log sheet. To maintain a constant incubator temperature, it may be helpful to remove the metal tube holder from the rotator while loading the samples. The tube holder is removed by carefully unscrewing the black knob in the center of the rotator and carefully removing the metal holder.
7. If using the bench top incubator, turn the incubator on (located on the bottom right side of the machine), push the left arrow twice until the screen reads: select program, choose P1, and then push start. Be sure to number beakers in order of the sample numbers they contain. Log info on the sheet provided next to the incubator.

*If time allows, label the TSB tubes with the sample numbers for the next morning.

SAMPLE PREPARATION (Day of procedure)

Samples usually need 2-6 hours of growth in TSB before they can be plated, so transfers must start early. Sterilize hood with disinfectant and UV it for 15 mins. During this time, set out your TSB tubes.

1. **Remove TSA plates the next morning. Sign out on incubator log sheet.**
2. **If TSB tubes have not been labeled, do this now.**
3. **Use a sterile inoculating loop or needle and aseptically transfer cells from the TSA plate to the corresponding TSB tube.**
4. **Incubate TSB tubes at 35°C for 2-6 hours.**

PREPARATION

Several materials must be brought to room temperature before you can proceed; set out disc dispensers (with discs), extra antibiotics, sterile transfer pipettes, sterile TSB flasks and Mueller Hinton plates a few hours before you plan on beginning.

1. Divide the antibiotics into 2 groups based on which stamper they will be used in. Bag these sets in Whirl-pac or Ziploc bags and set aside.
2. Autoclave swabs and 13X100mm tubes (cuvettes: 7 per self-seal bag) for 15 minutes at 121°C on a gravity cycle.
3. Cut parafilm into 1-inch square pieces and place into a large, empty weigh boat. You will need at least one square per sample, with extras for duplicates and mistakes.

4. Make sure you have enough cleaning supplies for stamper as outlined in the earlier Quality Assurance/Quality Control section.
5. Each tube in the shaker will have two plates for each drug panel; 1 and 2. Make sure plates are free of excess surface moisture. Place in incubator (35°C) or biohood with lids ajar about 10-30 minutes for moisture to evaporate (M2-A9, p8). Label the side of the bottoms of the plates with sample ID and number (1 or 2). For each 10th sample, label duplicate plates (1 &2) for that sample number. For example, if you have 50 samples, you will have 5 duplicates. You can label duplicates as the same sample number with "DUP" after it. Invert plates and bag them until needed. Label bags in order of sample numbers. Plates are usually bagged with 10 samples and their duplicate. Prepare duplicates of each of the controls (not to be counted as the 10% of samples) to ensure that one of the antibiotics does not fall off of a control, making that control void.
6. Turn on the spectrophotometer and set transmittance to 625nm. Let it warm up for 1 hr. Always ensure the spectrophotometer is level.
7. To calibrate:
    a. Use a 13x100mm tube (cuvette) filled with reagent water as the blank. Wipe outside surface of tube with a Kimwipe and cover with parafilm. Tube must be smudge free when placed into the spec. Put the blank into the spec and put the cap of the spec down. With the Milton Roy Spectronic 20D+, use the right knob to set the transmittance to 100%. Once it reaches 100%, set the mode to absorbency. The spec. should now blink 1.999. With the Thermo Spectronic Genesys 20, simply put the blank in and hit the "0 ABS/100% T" button and it will read "setting blank" until done.
    b. Leave on absorbency mode. Vortex McFarland turbidity standard No. 0.5. Wipe outside surface of tube with a Kimwipe and place into spec. Absorbency should be between **0.08 and 0.10.** Blanking with pure water is necessary to ensure the McFarland standard is within guidelines.
    c. Since the actual samples will be done with TSB, it is necessary to blank the spectrophotometer again. Repeat Step 7a with a cuvette tube of uninoculated TSB using a sterile transfer pipette.
8. Place samples, TSB flasks, swabs, transfer pipettes, plates, spectrophotometer tubes (cuvettes), extra test tube racks and parafilm in the biohood. Plug vortex in and set up where convenient.

PLATING SAMPLES

1. In the biohood, use a transfer pipette to transfer small amount of TSB (~3-4 ml) into cuvette. (You want enough liquid in the cuvette for the spectrophotometer to be able to pick up an absorbency reading.) Use a new pipette to transfer 2-3 drops of sample (from inoculated TSB tubes) into cuvette. Place parafilm over top of tube to seal, making sure to only let the side resting against the parafilm paper to rest face down towards the sample. If anything else comes into contact with the side of parafilm

resting against the parafilm paper, the sample could become contaminated during vortexing. Vortex and wipe tube with a Kimwipe. Place in spec and read absorbency. Absorbency should be between 0.08 and 0.10. If too high, carefully remove parafilm and aseptically add TSB from flask with transfer pipette. Parafilm, vortex, wipe tube, and read in spec again. If too low, add cells from sample tubes with transfer pipette. Parafilm, vortex, wipe tube and read again. After a few rounds of this, one will get a feel of the ratio of TSB to drops of sample, which is dependent upon the turbidity of the inoculated TSB samples. Theoretically, most of the samples should be approximately the same turbidity since they were all incubated for the same amount of time. It is best to do the controls first.

2. No more than 15 minutes after proper absorbency is reached place sterile swab in broth (M2-A9, p10). Rotate swab on side of tube to remove excess inoculum. If before swabbing, there is excess condensation on the plate, obtain a sterile swab and dab off excess moisture. Multiple swabs may be necessary if plate is too moist. Inoculate Plate 1 by streaking the swab over the entire agar surface (referred to as "complete lawn.") Repeat two more times rotating plate 60 degrees each time. Set the plate aside, invert and begin to stack them.
3. Repeat Step 2 for Plate 2. It may help to keep Plates 1 and 2 in separate stacks.
4. Allow broth to absorb 3 to 5 minutes (but no longer than 15 minutes) on the MHA plates before dispensing discs (the apparatus is referred to as disk tampers or stampers). To stamp the plates, place the disk stamper over the sample with the lid off and media side up. Make sure all the antibiotic sleeves are in the correct positions and the switch is in the "lock" position. Push the lever (top of apparatus) down carefully and steadily to ensure proper release of the antibiotics. Stamp Plate 1 with the stamper loaded with antibiotic group 1. Stamp Plate 2 with the stamper loaded with antibiotic group 2. Leave the plates right-side up under the hood for least 5-10 minutes so that the disks may set onto the media. In case not all of the disks come out simultaneously, flame sterilize forceps and use these to remove the proper undispensed antibiotic from the tamper and place in correct position on plate. Do not slide the antibiotics across the media surface when manually placing them. This could affect the results of the antibiotic resistance analysis.
5. Carefully remove plates from hood without disturbing the antibiotics. Carefully invert plates and place in 35°C incubator for 16 to 18 hours and log on the sheet provided. It is necessary to use extreme caution when doing this because if one antibiotic out of the whole set (usually 20) falls off, the entire sample is unusable for that day. The entire panel of antibiotics must be performed the same day per sample for accuracy and precision purposes.

READING PLATES WITH BIOMIC

BIOMIC is a computer based plate analyzer. The plate is photographed and zones are measured and interpreted by the computer.

1. Open BIOMIC (it has a biohazard symbol as its icon.)
2. Read the control plates first. Click on "New QC," which stands for Quality Control. Fill out Organism (drop down to proper species and ATCC number of the control organism), Initials (your initials), and Drug Panel (group 1 or 2). Ensure the test date is correct. This program walks one through the process with directions in the left menu panel. Open the drawer and line up the proper antibiotic with the arrow in the drawer. Click "Read plate", make sure all the antibiotics are in the proper place as the computerized zone diameter circles (adjust zones accordingly if need be), and click "View results". If the controls were done properly, all of the fonts should be green and say "OK" under the Quality column. Sometimes it says "N/A" for certain antibiotics for certain control organisms. If the control is within correct specifications, print test (if necessary), and hit "New QC" and repeat step 2 for both panels of all the control organisms. If all control organism QC data is satisfactory, it is not necessary to analyze the duplicates of the control organisms. The purpose of the duplicates of the control organisms is to ensure either the original or the duplicate came out with all 20 antibiotics in the proper place.
3. For the regular samples, click "New Specimen Test".
4. Fill out Specimen #, Technician (you), Supervisor, Specimen Type (e.g., stool), Organism Group (e.g., gram negative enteric), Organism (e.g., *E. coli*), Drug Panel (group 1 or group 2), and any Comments about the appearance of the plate.
5. Open drawer and place the plate on the reading tray making sure that plate is lined up correctly and correct Drug Panel was selected. The computer will prompt you on the proper way to place the plate, but if it is a group 1 antibiotic then the orange arrow on the reading tray must be lined up with AMC 30, if it is a group 2 then it must be lined up with CZ 30.
6. Click "Read Plate".
7. Observe results to ensure that all zones were read properly. Adjust as necessary.
8. Print page if necessary, click "Save" (the program usually saves automatically anyway, but just to be safe) and "Start New Test".
9. If at anytime something is not right, click "Discard test" and start over. Returning to the Main Menu at anytime leads to other menu options as well.
10. Once a sample has been run, it can be accessed under "Current Batch." Double clicking on the sample number allows access to the sample. There are colored tabs at the bottom left of the screen. If a sample ID was typed in wrong, it can be changed under the "Information" tab.
11. It is vital to make sure both plate 1 and plate 2 of each sample are available for analysis. If one is not, then the other should not be read either. Both drug panel sets must be ran and read in the same day to be valid. Once all of the plates have been read for the day, they need to be placed into triple bagged biohazard bags for disposal. No more than approximately 30-40 plates should be placed into a triple-bagged container due to the large volume of MHA that will melt during autoclaving. The bags should be secured shut and have autoclave placed on it with room number, initials, and date written on the tape with Sharpie. All bags should be autoclaved within one week.

MAINTAINING THE DATABASE

It is very important for the database of information to be maintained. It is also important to keep a hard copy (print out) of all data. BIOMIC will automatically backup any data once per day. Once you are done with a batch you need to send the current batch to the past. Do this by scrolling down the current batch, highlighting the last entry, right click, choose select all, right click again, then choose move to past. BIOMIC will automatically save a back up to the hard drive one time each day as you close out of the program. Occasionally, a backup copy (cd or flash drive) must be resaved to keep the raw data updated.

IMPORTING DATA INTO EXCEL

1. Open the BIOMIC program.
2. Click Transfer in the top menu toolbar.
3. In the dropdown menu, click “Data Export”.
4. It gives a test date range. It is important to note here that one can only pull by test date range, not by sample type or ID numbers. If a large amount of data is pulled, it may take a long while, and it is recommended to do it by smaller test date ranges. Enter the test date range required.
5. Click all pertainable checkmark boxes next to the fields required. SIR interpretation refers to “Susceptible, Intermediate, or Resistant” zone diameters. Test Date, Specimen Type, Drug Panel Name, and Zone Diameter are almost always recommended.
6. Click Begin Export. This will prompt a Save window to come up. The file is initially saved as a text file (*.txt) and needs to be named appropriately in the correct file. Click save when ready. The Save window will go away now. When it is done, click Okay in the original window.
7. Close out the BIOMIC program.
8. Open Microsoft Excel.
9. Click Data in the menu toolbar.
10. In the dropdown menu, click Get External Data. From the side menu, click Import Text File.
11. An Open File window will come up. Find the text file just saved in the appropriate save location. Double click on file or click Import.
12. The Text Import Wizard window will pop up. It should say Step 1 of 3. Click Delimited, and start window at Row 1. Hit Next. Under Step 2, click the Tab and Comma boxes only. Hit next again. Under Step 3, it should be clicked on General, and one should only have to hit Finish.
13. Then, it can either be opened in the existing worksheet or a new worksheet. Hit Okay.
14. The data should appear in columns; if not, start over. It is important to note that the data becomes imported as all of one drug panel in rows followed by the other drug panel underneath. Scroll down halfway to get to the other drug panel. It is usually desired to line up the drug panels so that all 20 antibiotic zone diameters or SIR interpretations are side-by-side next to the sample number. It takes very careful and meticulous cutting and

pasting to make sure that all of the data of a given sample stays together. Any minor mistake will have detrimental effects on the statistical analyses performed.

15. Once the required database is complete, it should manually be checked, zone diameter by zone diameter of every single sample against the printed results, which should be kept in three-inch binders in the lab or the lab coordinators office. Ideally, and at the discretion of the project manager, the numbers should be completely checked by two separate persons to be absolutely sure of accuracy of data.

TAMU-CC Biolog Procedure for *Enterococcus sp.* samples

Note: This procedure only outlines the proper analysis of *Enterococcus sp.* samples. Refer to the Biolog procedure binder for information about other types of samples.

Day before running samples:

1. Always use BugB medium and label plates clearly. If transferring from another plate, pull sample from an isolated colony. If transferring from another plate, pull sample from an isolated colony. If transferring from TSA slants, the culture should be pure. Place bacteria in the center of plate and do a triple lawn to evenly distribute cells.
2. Incubate BugB plates for 16-24h at 35°C.
3. Autoclave swabs and pipette tips.
4. Prepare inoculating fluid (20mL/tube, autoclave on a liquid cycle for 30 minutes)

Day of procedure:

1. Remove inoculating fluid tubes and microplates from refrigerator and allow them to reach room temperature.
2. Remove BugB plates in sets of 20 or 40. Label microplates accordingly and add a duplicate microplate every 20 samples
3. Turn on turbidometer and allow it to warm up for 10-15 minutes.
4. Calibrate turbidometer. Manually mix (tilt back and forth) a tube of inoculating fluid and place in the turbidometer. Set transmittance to 100%. Remove IF tube. Manually mix turbidity standard and place in the turbidometer. It should read 20% for the GP-Coc (Gram positive coccus) standard. Get as close to the targeted amount as possible, making sure that the value is within at least ± 5.
5. Wipe a tube of inoculating fluid carefully with a kimwipe and place in the turbidometer. The reading should be right at transmittance at 100%. If not, set to 100% and recalibrate with standard.
6. Open a new tube of thioglycolate (an anticapsulating agent). To do this, hold reagent dropper upright and point tip away from yourself. Squeeze middle gently once with thumb and forefinger to crush ampule inside the dropper.
7. Dispense 3 drops of thioglycolate into inoculating fluid. Do not use more than 3 drops per 18-20mL of fluid.
8. Moisten a sterile swab with inoculating fluid. Roll swab over the colonies rather than sliding across them. Be sure not to pick up any agar. Twirl the swab against the inside surface of the tube (above the fluid line) to gently break up clumps. Place swab in fluid. Swirl swab in fluid with a turbulent vertical motion to the bottom of the tube to create a uniform suspension, avoiding the sides of the tube. Cap tube tightly and invert tube 5 times to evenly distribute the bacteria. Do this carefully. Inoculum must be homogenous and free of clumps. If bubbles appear, wait for them to settle, or the reading will be inaccurate.
9. Read turbidity. It should be within ± 2 of the turbidity reading of your standard. If it is too high, add more bacteria using the procedure above. If it is too low, add more inoculating fluid with a sterile disposable pipette. Invert tube 5 times and read again. Repeat as necessary.

10. Once turbidity is in range, pour inocula into a reagent reservoir. Add tips to micropipette. It should be set to 1250µL (should be program 1).
11. Place pipette tips into reservoir and press "Fill." Inocula should be drawn into tips.
12. Align tips with first row of microplate and press gray button on the handle. Repeat this procedure for other rows. When you run out of fluid, press "Purge" button. Place micropipette tips over reservoir and push gray button to release fluid.
13. Repeat procedure until all rows are filled.
14. If any microplate wells are not full, fluid can be added using a sterile disposable pipette. Any overflow should be removed with a sterile swab.
15. Place lid on microplate and incubate at 35°C for 16-24h. Record log in information on the incubator log sheets.

Reading plates:

1. Open the Biolog 420 program and under INPUT screen select
    a. reader
    b. MicroStation2
    c. Comport 1
2. For each plate, fill in the following data
    a. Plate Info (pull-down menus—info is required for plate reading)
        i. Plate type (GP)
        ii. Strain type
        iii. Incubation time
    b. Plate Info (defined by user—optional)
        i. Sample number, Strain name, Strain number, Other
3. Plate reader must be on. After turning on, the plate reader will self-calibrate. After the self-calibration is over the reader will beep and the screen on the plate reader will say ready.
4. After self-calibration is complete, click the initialization button once. Initialization should be complete in a minute or two. The reader ready should change to yes on the computer monitor.
5. After initialization is complete, remove microplate lid and insert into reader snugly.
6. Click read?
7. After reading, a circle with a horizontal line through it means the well was negative and a circle with a plus sign means the well was positive.
8. The id is based on a progressive database which is based on the number of reactions in the plate; the specific pattern is what the mismatches are based on and the v. current microplate gives an idea where mismatches come from

Things to Remember:

- Keep turbidity standards out of light. Put them away as soon as you are finished with them.
- Cap inoculating fluid tightly to avoid spills during mixing.
- Cleaning micropipette tip holders with ethanol helps is you are having trouble releasing tips.

- Check expiration dates for all materials used